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MOULD GROWTHS UPON COLD-STORE MEAT.

By F. T. Brooks, M.A. (University Lecturer in Botany, Cambridge, and Food Investigation Board), and C. G. Hansford, B.A. (Food Investigation Board).

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I. INTRODUCTION.

An investigation of the mould fungi which contaminate chilled and frozen meat was commenced in 1918 when considerable quantities of imported meat were found to be damaged in this manner, probably chiefly on account of the abnormally long periods of storage which war conditions necessitated. Since then, numerous specimens of mould growths upon cold-store meat have been examined, and investigations have been made concerning the conditions under which these moulds develop. In 1921 a report (3) upon the "Black Spot" of chilled and frozen meat was published (Special Report No. 6, Food Investigation Board), and the present account contains a description of the other moulds which have been encountered during the course of the work*. These researches have been carried out under the auspices of the Food Investigation Board of the Department of Scientific and Industrial Research.

With one exception, all the fungi described have been repeatedly observed during the last three years upon cold-store meat, and in view of the multiplicity of fungoid forms, it is somewhat remarkable that those which occur upon meat are so few in variety. Our observations agree well with those of Massee (12), but he described in detail only *Cladosporium herbarum*, the cause of "Black Spot." Some of the other forms he mentioned, e.g. *Penicillium glaucum*, *Mucor mucedo* and *Mucor racemosus* we have seen, but *Oospora carneola*, *Verticillium lateritium*, *Penicillium candidum* and *Phycomyces nitens* we have not seen, notwithstanding repeated search. Klein (10) referred

* We are indebted to Mrs M. N. Kidd for assistance during the earlier stages of this investigation.

the cause of "Black Spot" to *Oidium carnis* but this is doubtless another, but incorrect, name for *Cladosporium herbarum.* Tabor (17) has recently contended that "Black Spot" of meat may be produced by many different fungi, but in our experience there is no satisfactory evidence for this statement. The fact that he has invariably failed to obtain growth from "Black Spots" shows that his methods are imperfect, and his contention that the "Black Spots" are due to the death of various mould fungi in the meat cannot be substantiated. In practically every case that came under our observation, the "Black Spots" on meat contained living mycelium which gave rise, under suitable conditions, invariably to *Cladosporium herbarum,* and to no other fungus. Furthermore, the only fungus with which we have been able to reproduce "Black Spot" on meat in cold storage is *Cladosporium herbarum.* It often happens that other mould growths of a more superficial character, such as *Penicillium* and *Mucor,* are superimposed upon "Black Spot," but in no case have they had anything to do with the very well-defined trouble of "Black Spot."

While this work was in progress, Monvoisin (13) in France, was making a similar investigation unknown to us. He published a short paper, the results of which agree in the main with our observations. The fungi he specifically mentions as occurring on cold-store meat are *Thamnidium elegans, Mucor mucedo, Rhizopus* sp. and *Penicillium glaucum.* At a much earlier date, Talayract (18) had recorded the occurrence of *Penicillium, Sporotrichum, Mucor* spp., *Dematium,* and pink yeasts upon imported meat which he had seen in the London docks, the *Dematium* mentioned here being doubtless *Cladosporium herbarum.*

Quite recently another French worker, Bidault (2), has published a brief account of the moulds of frozen meat. The forms of commonest occurrence according to him are much the same as those found by us, but in our experience *Botrytis* spp. and *Stysanus stemonitis* have not been seen. On the other hand, some species commonly found by us are not recorded by him. Details of experiments on the growth of these moulds at low temperatures are not given, but he states that *Chaetostylum Fresenii* (= *Thamnidium chaetocladioides*) and *Hormodendron cladosporioides* (= *Cladosporium herbarum*) will grow slightly at − 10° C., and that others will grow between − 6° and 0° C.

It was shown in the previous report (3) that the "Black Spot" fungus possessed the remarkable property of growth at − 6° C. Another of these meat moulds, *Torula botryoides,* was also found to develop at this low temperature, and more recently certain others have given evidence of slight power of development at − 6° C. All these forms grow readily at 0° to 2° C., and perhaps

others that have not yet shown growth at – 6° C. will be found capable of development between – 6° and o° C.; this will shortly be tested. Monvoisin(13) states that mould spores (species not stated) will not germinate under cold-storage conditions, but that these forms, if allowed to germinate for sixteen hours at ordinary temperature, will continue their growth in the cold-store and produce new sporing bodies in the course of four to five months. Monvoisin does not state definitely the forms with which he experimented, but he implies that they were the species mentioned above. Our own results show that the spores of *Cladosporium herbarum* germinate even at – 6° C., but that subsequent growth is more rapid if germination has taken place for a short period at ordinary temperature. Failure to get some of the other species to develop at – 6° C. may perhaps be due to the conditions of humidity being different from those in Monvoisin's experiments. There is no doubt that some of these mould contaminations are due to the meat being exposed to temperatures above o° C., especially a few degrees higher than this, as at more enhanced temperatures bacterial growth is so vigorous that mould development is inhibited to a great extent. On the other hand, it has been shown that prolonged storage may induce the formation of certain of these mould growths, e.g. "Black Spot," even at several degrees below o° C. Shorter storage at a temperature just below o° C. will also give opportunity for the development of some of these moulds.

The spores of all mould fungi found on cold-store meat retain their vitality for long periods, several, notably *Cladosporium herbarum*, *Penicillium expansum* and *Thamnidium* spp., remaining alive after being subjected to a temperature of – 6° C. for two years. A period of three years at this temperature has, however, killed even these forms unless growth has already occurred in the cold store.

Other common mould fungi also retain their vitality for long periods at low temperatures. For instance, spores of *Botrytis cinerea*, *Aspergillus niger* and *Acrostalagmus cinnabarinus*, germinated after being kept for a year on the surface of culture media at – 6° C., but spores of *Cephalothecium roseum*, *Fusarium coeruleum* and *Rhizopus nigricans* were killed under these conditions. It is noteworthy that prolonged exposure to cold retards the rate of germination of mould spores, and that young mycelia are more quickly killed by low temperatures than are spores. Recent research has indicated that even thin-walled fungal spores retain their vitality for much longer periods than was formerly supposed, and the present results confirm this.

In the course of this work it has been necessary to undertake a systematic study of many strains of certain of these fungi

which occur upon meat and upon vegetable substrata, and the observations upon the differences between these closely related strains are recorded here.

Several of these fungi are of common occurrence as moulds upon different kinds of organic matter, e.g. *Cladosporium herbarum*, but three are apparently new to science. Some occur commonly as moulds upon vegetable debris within and in the vicinity of abattoirs, and it is likely that all so occur. The similarity of the types of mould occurring on meat imported from different countries is very striking, and point to the cosmopolitan distribution of these fungi.

II. *CLADOSPORIUM HERBARUM.*

This fungus has been shown to be the cause of the trouble known in the frozen meat trade as "Black Spot." Several strains isolated from different kinds of meat have been proved to possess the power of growth below freezing point. Strains of this fungus have also been isolated from other sources, chiefly vegetable substrata, and some of these are also able to grow at $-6°$ C. For comparison, cultures of various species of *Cladosporium* were obtained from the Centraalbureau voor Schimmelcultures, Amsterdam. These were:

C. herbarum (No. 43) *C. epiphyllum* (No. 44)
C. Aphidis (No. 42) *C. carpophilum* (No. 47),

also *C. butryi* and *C. cucumerinum*, neither of which could be induced to fructify. In this connection it is noteworthy that other forms occasionally degenerated in the course of the work when cultivated for several generations on meat extract-peptone-agar.

In a paper on the mould-growths of frozen meat, Monvoisin (13) makes no special mention of *Cladosporium*. Bidault (2) records both *Cladosporium herbarum* and *Hormodendron cladosporioides* on frozen meat, but, for reasons which will be given later, we consider these to be identical.

In connection with the identification of the fungus causing "Black Spot" of meat, it was necessary to examine critically many closely related forms of *Cladosporium*. One of the results of this investigation has been to show that many so-called species of *Cladosporium* are not really specifically distinct from *C. herbarum*, but are only slightly different strains of the same fungus.

It is not only on meat that *Cladosporium herbarum* causes black spots. In September 1921, one of the writers saw dead fronds of the seaweed *Laminaria digitata* covered with black spots which to the naked eye appeared indistinguishable from

the "Black Spot" of meat (Fig. 1). Upon isolation, it was found that the cause of the black spots on the seaweed was also *Cladosporium herbarum*. The texture of such a seaweed is not unlike that of the connective tissue of meat upon which black spots are most prone to develop, and, as in the latter, the discoloration is due to the dark hyphae of the fungus ramifying in the tissues.

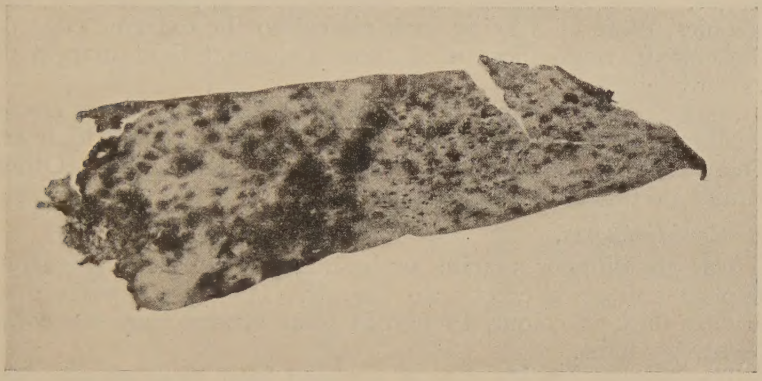


Fig. 1. "Black spot" on *Laminaria digitata*.

In view of growth proceeding at as low a temperature as $-6°$ C. it is clear that the sap of this fungus must possess a high osmotic concentration. The hyphae are narrow and it is difficult to observe plasmolytic phenomena in them, but immersion in a 15 % solution of calcium chloride undoubtedly causes plasmolysis while immersion in a 10 % solution is without effect.

The following is chiefly a systematic comparison of the characters of the different forms of *Cladosporium* isolated from meat and from vegetable sources.

I. Morphological characters, etc.

The following remarks apply to all strains used during this work. Each strain is denoted by a letter or number.

1. *Germination of Spores.*

The spores swell and then put out one, or sometimes two, germ tubes. These usually give rise to a branched mycelium, but occasionally when the conidium has given rise to two germ tubes, one of these forms a short conidiophore of the usual *Hormodendron* type.

2. *Hyphae.*

The hyphae are uniform in size and appearance in all strains, varying from 3–7 μ in diameter, and are septate at short intervals. In colour they vary from hyaline to almost black, according to age and strain. The length of time taken by the hyphae to turn dark olive varies with the strain, some, e.g. strain *S*, taking a very short time, others, e.g. *Z*, taking much longer. This influences the macroscopic appearance of the colonies, those of *S* being dark almost to the extreme edge of the colony, while those of *Z* show a distinct light margin of varying width.

Some strains, particularly *Z*, 60, 119A, when grown on Dox's medium, show fine, hyaline hyphae coiled in a peculiar manner. These arise as lateral branches of the normal hyphae. Other strains do not show this peculiarity.

3. *Conidiophores.*

The conidiophores arise as branches from the vegetative hyphae and grow erect into the air. They are septate and usually dark in colour, though in some strains they are comparatively light.

The present strains can be grouped into three classes, according to the length of the conidiophores, thus:

(*a*) Short conidiophores (less than 100 μ)—*Z*, 43, 108A, etc.
(*b*) Medium ,, (100–250 μ) —44.
(*c*) Long ,, (over 250 μ) —*S*, 110.

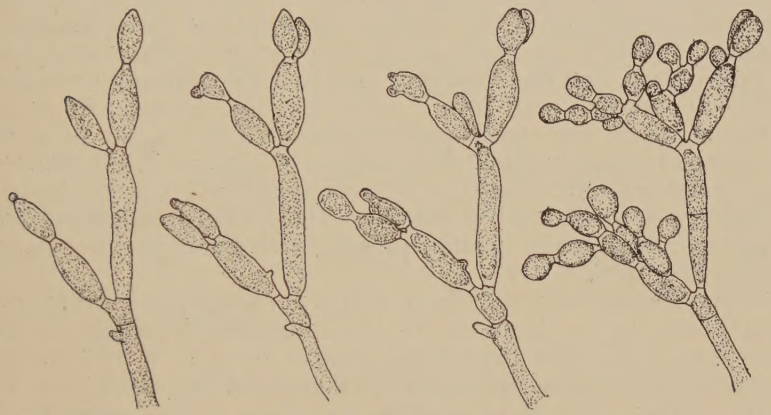
These classes are arbitrary and all gradations occur between the shortest and longest. This classification is based on a series of cultures in Petri dishes, on potato agar in the light at laboratory temperature. The width of the conidiophore is approximately that of the hyphae from which it arises.

The differentiation of the conidiophores from the vegetative mycelium varies in different strains. In *S*, the conidiophores are very distinct from the mycelium, whereas in *Z* the conidiophores are not differentiated. This distinction is more marked in those strains with long, unbranched conidiophores. In some strains the sterile part of the conidiophore is much branched, thus resembling the vegetative mycelium. In strain *S* the conidiophores are invariably unbranched, except at the apex where the head of conidia is formed.

4. *Formation of Conidia.*

The tip of the conidiophore is cut off by a wall to form the first conidium, this being invariably of the large type. The cell of the conidiophore immediately behind the first conidium may or may not grow out to form a second conidium, lateral to the

first. In nature each conidiophore usually bears a few large spores, but under culture conditions these large spores bud forth, giving rise to chains of smaller spores, the so-called *Hormodendron* stage. The youngest conidium is that at the distal end of the chain, the large conidia next the conidiophore being the oldest. Each conidium of a chain arises as a small bud on that immediately behind (cf. Fig. 2).



(1) 10 a.m. Mon. (2) 3.0 p.m. Mon. (3) 6 p.m. Mon. (4) 9.0 a.m. Tues.

Fig. 2. Formation of conidia of *Cladosporium herbarum*. (Hanging drop culture). × 900.

While still quite small, this bud is cut off by a membrane from the parent conidium, and continues to enlarge. The membrane between the young conidium and its parent is thickened from both sides simultaneously with the growth of the former, until finally, under certain conditions of microscope illumination, it appears as a small intercalary piece (cf. Fig. 3). On separation of the conidia, these "intercalary pieces" break asunder, along the line of the original membrane between the two conidia. Thus the majority of the conidia when separated, show a small stalk at each end, giving them a somewhat lemon-shaped appearance.

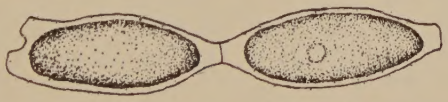


Fig. 3. Conidia of *C. herbarum* showing thickened end walls. × 3100.

The method of spore formation is the same in all the present series of forms.

5. *Conidia.*

As mentioned above, the conidia in artificial cultures differ considerably from those formed in nature. Upon vegetable debris in nature, the conidia are almost entirely of the large variety, while in cultures the large conidia are masked by the enormous numbers of small conidia formed by the process of budding described above. In culture, the conidia are in long branched chains, forming a more or less dense head at the end of the conidiophore. The number and length of these chains of small conidia is an index of the amount of budding that has taken place, and has a marked effect on the appearance of the conidiophore as seen intact under the microscope. In some strains, as *S*, the conidiophore terminates in a dense mass of chains of small conidia, while in other strains, as *Z*, the process of budding is much restricted, the conidiophore being terminated only by a small head of conidia. The budding influences the percentage of large conidia present, this being much greater in *Z* than in *S*.

The conidia vary greatly in size, from small spherical spores of about 4 μ in diameter, to large cylindrical conidia up to 25 $\mu \times$ 4–5 μ. The large basal conidia of the "head" merge gradually into the small spherical conidia of the distal parts of the chains. The large conidia are more or less cylindrical, tapering slightly at the ends, and may be 1–3 times septate, the septation being more frequent in old cultures.

According to Saccardo(15) and Rabenhorst(14) the large conidia of *C. herbarum* are constricted at the septa, but while this constriction is common in nature, though by no means universal, it is exceptional in artificial cultures, and is not characteristic of any particular strain. The same authors state that the walls of the conidia of a form (*Vincetoxici*) of *C. herbarum* are granular to echinulate, but in our cultures indications of a roughness of the conidial walls were very rare, although the walls of the conidia originally formed in nature by some strains were certainly rough.

The colour of the conidia as seen under the microscope varies with their age, the largest (i.e. also the oldest) conidia being darker in colour than the small conidia of the distal ends of the chains. In some strains, as *Z*, the conidia never become very dark in colour, while in *S* they become almost black. Every intermediate between these two extremes is present.

A minor point of difference between the two extreme strains *S* and *Z* is that the latter never formed truly spherical spores, while in *S* the fourth or fifth conidium from the base of the "head" was practically spherical.

II. Macroscopic Characters of Strains.

Although the various isolations approach each other so as to be almost indistinguishable under the microscope, to the naked eye there are considerable differences between them as indicated in the table, p. 125. Few cultures of the fungus isolated during the present work were absolutely identical in appearance. The strains were grown on Dox's medium with the full amount of sugar (30 gms. per litre). This medium proved to be the best for differentiating between the various strains, the cultures being compared at the end of two months' growth. Growth on this medium was luxuriant, and two characters were used to differentiate the strains:

(1) Colour.
(2) Texture of colony—whether "woolly" or not.

The "woolly" texture of some strains is due to the aerial growth of sterile mycelium—the hyphae being often very fine and almost hyaline—forming a layer over the conidiophores. The characters of the various strains were much the same when grown upon steamed potato.

III. Physiological Characters of Strains.

1. *Influence of Temperature.*

The approximate range of temperature within which each strain would grow was determined. The different strains show considerable divergence in this respect, and the grouping of the strains, according to this character, does not follow the arrangement according to other characters; the table given below is an attempt to classify them according to both.

As already recorded, some strains proved capable of growth below freezing point, at − 6° C., and probably others which would not grow at this temperature could develop at a slightly higher temperature, as all the strains isolated from "Black Spot" on meat in cold storage grew well at 2° C. Temporary freezing up to a month at − 6° C. had no effect on the rate or percentage of germination of the spores subsequently tested at room temperature, but prolonged freezing at − 6° C. eventually killed the spores of most strains, except such as proved capable of germination and growth at this temperature.

Temporary freezing (up to three weeks) of the vegetative mycelium of strain *S* had no permanently adverse effect, beyond greatly retarding growth.

2. *Influence of Light.*

With a view to the possibility of distinguishing forms of *Cladosporium* from those of *Hormodendron*, according to the

method of Schostakowitsch (16), a series of cultures was made in Petri dishes and exposed to illumination from one side only. A few strains showed a positive reaction to light, the conidiophores bending towards the source of light. The strains showing this characteristic were *S*, 102D, 103B, 113, all of which were originally isolated from meat in cold storage. Several other strains isolated from similar sources showed no reaction to light.

3. *Influence of increased concentration of mineral salts.*

Schostakowitsch (16) states that he was able to distinguish the two forms *Hormodendron* and *Cladosporium* by their characters when grown in solutions containing a high concentration of potassium nitrate. A series of cultures of the two strains *S* and 43 was made in a nutritive solution, to which various amounts of KNO_3 were added. Both strains grew in all strengths of KNO_3 up to saturation, and the differences between the two strains were much less in the more concentrated solutions than under more normal cultural conditions.

IV. Relation of *Cladosporium* to *Hormodendron*.

According to Bancroft (1) conidia of the *Cladosporium* type when germinated at a low temperature (below 56° F.), form a mycelium which produces other conidia of the same type. If germinated at a higher temperature (60° F. or above) he states that the conidia formed belong to the *Hormodendron* type. In the course of the present work it was found that Bancroft's statements could not be confirmed for any of the strains used. The "*Hormodendron*" stage was produced in every culture grown at low as well as at high temperatures on both solid and liquid media. Even at − 6° C. "Black Spot" produced by artificial inoculation of meat gave rise to many *Hormodendron* spores, although the proportion of large spores of *Cladosporium* type was greater than at ordinary temperature.

On examination of the present series of cultures it was found that the distinction between *Cladosporium* and "*Hormodendron*" was merely a question of the amount and character of the budding of the first-formed conidia as described above, and that the two forms merged gradually one into the other.

It is noteworthy that in nature the "*Hormodendron*" type is less common than the *Cladosporium*. In our experience the strains isolated from the latter type do not differ more from those isolated from the former type, than they differ among themselves. Thus the claim of Schostakowitsch (16) that he was able to differentiate the two types by

(*a*) the positive heliotropism of *Hormodendron*,

(*b*) the rough conidial walls of *Cladosporium*,
(*c*) the production of conidia by *Hormodendron* in a higher concentration of KNO_3 than *Cladosporium*,

is not supported by the present work.

No indication of any connection of *Cladosporium herbarum* with *Dematium pullulans* as mentioned by Delacroix and Maublanc (4) was observed in the course of this work, neither was there any evidence of a perithecial form as described by Janczewski (7-9).

V. Discussion.

Lindau in Rabenhorst's *Kryptogamen-Flora* in a note on the genus *Cladosporium*, mentions the great range of the species *C. herbarum*, and the consequent difficulty in diagnosing both this species and the whole genus. In a note on the "species" *C. epiphyllum* he expresses a doubt as to whether it is distinct from *C. herbarum*, and says that the two are often confused.

In the present work it was impossible to distinguish the Dutch culture of *C. epiphyllum* from some of the other forms. The whole of the present series of forms with the exception of No. 47 (*C. carpophilum* from Holland) were so closely allied in cultural and microscopic characters that separation into distinct species was impossible.

In Rabenhorst's *Kryptogamen-Flora* and also in Saccardo's *Sylloge*, the various "species" of *Cladosporium* are grouped more or less according to the substratum upon which they occur. This is very unsatisfactory, especially in view of the enormous range of hosts of the single species *C. herbarum*, and it is more than probable, in the light of the present work, that the great majority of the "species" described in these works ought to be included in *C. herbarum*. The diagnoses of many of the species are so vague that they cannot be taken seriously into consideration. Certainly, as a result of the present work, the two "species" *C. epiphyllum* and *C. Aphidis* must be included as synonyms of *C. herbarum*. These forms were sent from the Centraalbureau voor Schimmelcultures, Amsterdam, and represent, in our opinion, merely strains of the species *C. herbarum*.

On the other hand, the culture of *C. carpophilum* from the same source differed so greatly from all other strains that it must be retained as a distinct species. Thus, in view of the present work, the species of *Cladosporium* should be revised entirely, and diagnosed afresh, not only on the basis of their host plants and morphological characters as found in nature, but also upon their cultural characters.

VI. Table of differences between strains of *Cladosporium herbarum.*

The various strains included in the table on the opposite page are arranged as far as possible as intermediates between the two extremes, *S* and *Z*. Many other strains are not included, on account of their extreme "woolliness" on all media, which rendered it difficult to group them in this series. The table illustrates the wide range of the single species *C. herbarum*. The classification is based upon the following points, which were selected as the most definite criteria:

(1) colour and texture of colonies on Dox's medium,
(2) ,, ,, ,, steamed potato,
(3) length of conidiophores,
(4) branching of "stalk" of conidiophore,
(5) whether "heads" of conidia are dense or not (i.e. an index of the relative amount of budding of conidia),
(6) temperature relations,
(7) reaction to light.

III. *SPOROTRICHUM CARNIS* n.sp.

This fungus was more frequently found upon all kinds of meat in cold storage than any other. It occurs in the form of innumerable white, slightly woolly patches, small in extent, and is the commonest form of "white mould" known to the meat trade. The growth of this fungus on meat is entirely superficial. It was present to some extent at any rate upon practically every sample of mouldy meat examined, although "Black Spot" caused by *Cladosporium herbarum* was sometimes more abundant. Talayract (18) mentions the occurrence of *Sporotrichum* upon chilled meat, but does not say what the species was.

Many slightly-differing strains were isolated from contaminated meat, but it is considered that all these belong to one species, a new one, *Sporotrichum carnis*. For comparison, cultures of *S. bombycinum* and *S. globuliferum* were obtained from the Centraalbureau voor Schimmelcultures, Amsterdam.

A form of *Sporotrichum*, indistinguishable from *S. carnis*, has recently been found to be of fairly common occurrence in slime fluxes of trees by Mr L. Ogilvy working at Cambridge, and one of us working in a Danish laboratory a short time ago encountered this fungus as a laboratory contamination.

Table of differences between strains of Cladosporium herbarum.

Strain	Origin	Macroscopic appearance on *Dox's medium* (two months' growth)	Length of Conidiophores	"Heads" of Conidia	Branching of Conidiophores	Range of temperature within which growth occurs*	Macroscopic appearance on potato chunks	Heliotropism
S	Meat in cold storage	Green brown, not woolly	Long	Very dense	Not branched	− 6° C. 20° C.	Almost black, not woolly	+ heliotropic
103B	,, ,,	,, ,,	,,	,,	,,	− 6° C. 20° C.	,, ,,	,,
113	,, ,,	,, ,,	,,	,,	,,	2° C. 20° C.	,, ,,	,,
50B	*C. herbarum*? from Miss Smith, South Kensington	,, ,,	Medium-long	,,	Occas. branched	2° C. 25° C.	,, ,,	Not heliotropic
102D	Meat in cold storage	,, ,,	Long	,,	Often branched	?	,, slightly woolly	+ heliotropic
114B	,, ,,	,, ,,	Medium-long	,,	,,	− 6° C. 25° C.	Grey-green, very woolly	Not heliotropic
112	,, ,,	Green brown, woolly	,, very long	,,	Branched	2° C. 20° C.	,, ,,	,,
110	,, ,,	Bright green, slightly woolly	Very long	Not as dense as *S*	,,	2° C. 20° C.	Almost black, slightly woolly	,,
31	Black Currant twig	Green-black, not woolly	Medium	Very dense	Much branched	2° C. 25° C.	Black, light margin, slightly woolly	,,
44	"*C. epiphyllum*" from Holland	Bright green, not woolly	,,	,,	Branched	2° C. 25° C.	Green, very woolly	,,
37	Honeydew in greenhouse	,, ,,	Short-medium	Not as dense as *S*	,,	2° C. 30° C.	Almost black, not woolly	,,
54	Tomato fruit	Green-black ,,	,,	Very dense	,,	?	Light green, very woolly	,,
42	"*C. aphidis*" from Holland	Green-grey, slightly woolly	,,	Lax	Occas. branched	2° C. 20° C.	Green-brown, slightly woolly	,,
43	"*C. herbarum*" from Holland	Green-brown ,,	Very short	,,	Not branched	2° C. 30° C.	,, not woolly	,,
55	Maize shoot	Green-brown, woolly	Short	,,	Occas. branched	2° C. 25° C.	Green, woolly	,,
62	Wheat spikelet	,, ,,	Medium	Dense	,,	?	Green-brown, slightly woolly	,,
60	Dead leaves	Light green-grey, woolly	Short	Lax	Not branched	2° C. 25° C.	Grey-brown, very woolly	,,
108A	Meat in cold storage	Green, very woolly	,,	,,	,,	2° C. 25° C.	,, slightly woolly	,,
119A	,, ,,	Black, light margin ,woolly	,,	,,	,,	?	Green, slightly woolly	,,
Z	,, ,,	Green-black, slightly woolly	,,	,,	,,	− 6° C. 25° C.	Green-brown, not woolly	,,

* The lower temperature is not necessarily the minimum.

I. Microscopical Characters.

The following remarks apply to all strains of *Sporotrichum carnis* used in this work, except where otherwise stated. The various strains are denoted either by a letter or a number.

1. *Germination of the spores.*

The spores swell considerably, each putting out one or two germ tubes, which give rise to a branched mycelium.

2. *Hyphae.*

The hyphae are very narrow, about 1 μ in diameter, and invariably hyaline. On some agar media the hyphae inside the medium differ from the normal type, being rather wider, and distinctly vacuolate. In the normal hyphae the septa are very obscure, but in these submerged hyphae the septa are more prominent. On agar the hyphae are usually straight, giving off branches almost at right angles to the parent hyphae.

The few strains which grow at 30° C. form peculiar stromatic colonies, consisting of swollen hyphae, the cells being very short and almost spherical. There is no penetration of the medium at this temperature as there is at ordinary temperatures.

3. *Formation of conidia* (Fig. 4).

(*a*) *Sporotrichum carnis.* Aerial branches arise from the vegetative mycelium, 30–50 μ in length. On these, other short branches arise, often in twos and threes, which may branch again. The branches are cut off by septa from the parent hypha, and the distal parts of the whole system of branches segment into short, cylindrical cells, 3–5 μ long. These cells form conidia, the apical portion of each swelling considerably, and their walls, especially the transverse wall at the base, thicken. Those cells of the conidiophore and its branches which do not form conidia disorganise, leaving nothing but the walls, which are almost invisible. The conidia are easily detached, and on mounting a portion of an old colony, a mass of spores is seen, with only occasional portions of hyphal walls.

(*b*) *Sporotrichum globuliferum* (from Amsterdam). As shown in Fig. 5, the conidiophore of *Sporotrichum globuliferum* differs greatly in appearance from that of the species isolated from meat. Long, aerial branches arise from the vegetative mycelium, many remaining sterile and contributing to the woolly appearance of the colonies, while others develop small groups of conidia at their ends and on short lateral branches. The conidia are both terminal and lateral on short sterigmata, those at the apex of the conidiophore being the youngest. This method of spore formation is distinct from that of *Sporotrichum carnis.*

4. *Conidia.*

The conidia of *S. carnis* vary greatly in size and shape. The majority, owing to their peculiar method of formation, are

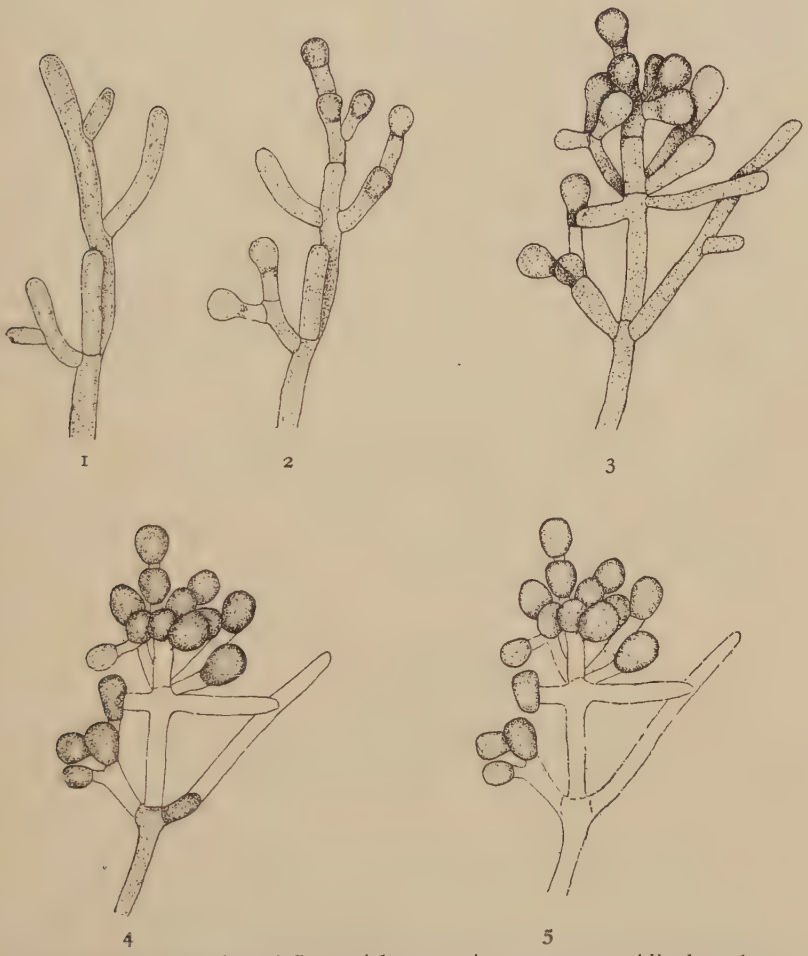


Fig. 4. Spore formation of *Sporotrichum carnis*. 1, young conidiophore from hanging drop culture, 48 hours old; 2, same, after a further 12 hours; 3, same, after a further 12 hours; 4, same as 3, 48 hours later; 5, same as 4, 48 hours later. × 2060.

somewhat pear-shaped, but others approximate to spherical. All the strains form hyaline conidia varying from 2–5 μ in length.

II. Macroscopic characters of strains of *S. carnis.*

The strains were cultivated on various media of which Dox's agar (containing half the usual amount of sugar), Sabouraud's glucose agar, and potato chunks were selected as best. On these media, especially on the second, the various strains, even when young, show marked differences. All are more or less white when young, but some show a peculiar yellow or orange discolouration inside the medium, which is absent from others. When the colonies are old, the aerial portions differ greatly in colour, varying in different strains from white to brown. Some strains are considerably more woolly in culture than others, especially on potato chunks.

III. Physiological characters (*S. carnis*).

(*a*) *Chemical.* The various isolations were grown in 4 % solutions of sugar to which peptone and litmus had been added.

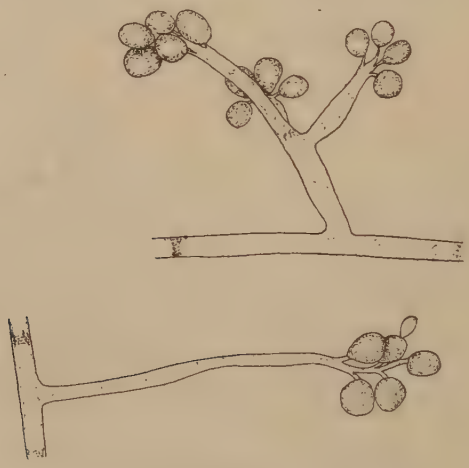


Fig. 5. *Sporotrichum globuliferum* conidiophores. × 2060.

They showed no great differences in their fermentation properties, all fermenting glucose, maltose, and laevulose, though the amount of acid produced varied to some extent. One or two forms also fermented sucrose. Some forms, especially those which showed a yellow discolouration of ordinary media, produced a yellow or orange pigment which diffused through the liquid. Three forms produced a dark-brown pigment instead of a yellow, and in one the litmus was bleached when the cultures were kept a month.

(*b*) *Temperature relations.* The forms were grown at different temperatures. Very slight indications of growth on artificial media at – 6° C. have been obtained in some strains after long periods, and as the majority of forms develop well at 2° C., it is likely that better growth occurs just below 0° C. than at – 6° C. The large white spots sometimes seen on meat have probably arisen through exposure to temperatures round about freezing point; there is no indication yet that such large growths would develop at – 6° C.

IV. Cultural differences of strains of *S. carnis.*

Scarcely any two isolations appeared identical in culture, but they can be arranged conveniently in four groups, according to their macroscopic appearance on Sabouraud's glucose agar, the medium on which they grow best. The microscopical characters of the strains are so similar as to be of no value for differentiating them.

1. *F Group*: comprising forms *F*, 121A, 104B, all isolated from meat in cold storage, and *L*, isolated from a Petri dish exposed in an abattoir in the Argentine.

This group is distinguished by its rather smooth colonies, which are not heaped up on the surface of the medium as in the next group, and by the development of a yellow pigment on almost all media used.

2. *W Group*: comprising forms *W*, 102E, 120B, 110A, all isolated from meat in cold storage.

These are very closely related and are characterised by their colonies being usually heaped up on the surface of the medium. In colour they vary from white to brown, and they produce no yellow pigment in the medium, as in the preceding group. Instead, in some liquid cultures they produce a dark brown pigment, diffusing through the liquid.

3. *"Woolly" Group*: comprising 118D, 120C, 103A, 101A, 104A, all isolated from meat in cold storage.

The extreme "woolliness" of these strains on all media distinguishes them from the other groups. The colonies are dirty brown in colour. These strains produce no yellow pigment in the medium, but some produce the dark brown pigment noted under the preceding group.

4. 119C *Group*: comprising 119C and 115G, isolated from meat.

On all media used these strains have very dense, smooth colonies, quite different from other groups. The colonies show

alternating coloured and white zones, the colour varying from pink to buff. On some media 115G develops a dark red colour.

It may be mentioned that *Sporotrichum globuliferum* differs both microscopically and macroscopically from all strains of *S. carnis*. On all media the colonies were light yellow in colour and extremely woolly, and in liquid cultures this fungus developed a yellow or orange pigment, which diffused into the medium.

V. Discussion.

The forms isolated from meat, although falling into the above groups may yet be classified as one species, as under the microscope no differences can be seen which would justify separation into distinct species.

As far as one can determine from the meagre diagnoses of other species of *Sporotrichum* given in Rabenhorst's *Kryptogamen-Flora* and in Saccardo's *Sylloge*, the present species differs from all others previously described. The diagnoses of the species given in these books are often so inadequate that identification is impossible, and unless re-diagnosis is possible it would be better to discard them. Diagnosis of such species should be based, not only on the characters exhibited when growing on the original substratum, but also on cultural characters on standard media under controlled conditions.

VI. Comparison with other Sporotrichums spp.

The diagnosis of the genus in Rabenhorst's *Kryptogamen-Flora* is as follows:

Hyphae: forming a "turf," septate or not septate, creeping or decumbent, irregularly but never verticillately branched; branches usually branched again.

Conidiophores: hardly differentiated, at most very like the ordinary side branches.

Conidia: lateral or terminal on the hyphae or on small branches, usually very numerous, may or may not have well-developed sterigmata, oval or spherical, hyaline or slightly coloured, very small.

In a note on the genus, Lindau says that "only in a few cases are the conidiophores erect, thereby being distinguished from the side branches; the spores are usually produced on the creeping mycelium, which soon disappears. Then the colony consists of masses of spores and remains of hyphae. The spores are produced terminally but through growth of the hyphae soon become lateral; the spores are often situated on small protuberances of the hyphae."

This method of spore formation is much the same as that

described for *S. globuliferum*, but is very different from that of *S. carnis*.

To this method of spore formation given in Rabenhorst, must be added a second, that of the present species as described above.

The diagnosis of this new species is as follows:

Sporotrichum carnis n.sp.*

Forming circular colonies, white, closely adpressed to the substratum.

Hyphae creeping, interwoven, branched, septate, septa very obscure, hyaline, 1–2 μ wide.

Conidiophores not well differentiated, much branched, hyaline.

Conidia formed laterally or terminally from slightly swollen distal cells of branches of conidiophores, hyaline, 2–5 $\mu \times$ 2–4 μ, oval-pyriform. The conidiophores soon disorganise after formation of conidia. In artificial culture the colonies may appear coloured, varying from pale yellow to dark reddish brown and may be compact or woolly.

Habitat on meat which has been kept in cold storage.

IV. *TORULA BOTRYOIDES*, n.sp.

This fungus was first isolated in May 1918 from a halibut which had been kept in cold storage. The fish was sent to one of the writers in connection with another enquiry then in progress; upon arrival it was apparently free from moulds. Within a few days, however, whitish fluffy growths began to appear upon it, especially around the mouth and gills, notwithstanding the fact that the fish was kept in a refrigerator below 0° C. except when being examined. The same fungus was subsequently isolated from beef, mutton, rabbits, and sausages which had been kept in cold storage. On meat it produced a greyish-white, rather woolly growth, quite distinct in appearance from *Sporotrichum carnis*.

I. Microscopic characters.

1. *Germination of spores.*

The spores swell considerably and produce germ tubes, which give rise to a mycelium of branched hyphae. In this connection

* *Sporotrichum carnis* sp.nov.

Coloniis candidis substrato arcte adpressis; hyphis repentibus, intertextis, ramosis septatis (septis valde inconspicuis), hyalinis, 1–2 μ latis; conidiophoris haud bene evolutis, valde ramosis, hyalinis; conidiis in ramulorum tumidulis apicibus pleurogenis vel acrogenis, hyalinis, 2–5 $\mu \times$ 2–4 μ, ovali-pyriformibus. Conidiophoris post conidia effecta mox dilabentibus. Coloniae in mediis nutrientibus cultae diverse coloratae, interdum pallide luteae interdum fusco-rubello-brunneae, et congestae vel flocculosae sunt.

Hab. Ad carnem in frigidariis asservatam.

We are indebted to Mr Gepp and Mr Ramsbottom of the British Museum for assistance in drawing up the Latin diagnoses.

it may be noted that no indication of the formation of a dense aggregation of cells was obtained, as described by Kr. Høye (6) for *Torula epizoa*, a common fungus on salted cod in Norway. Even when sown in fish extract with 10 % salt added, the spores germinated in the usual manner.

2. *Hyphae.*

The hyphae are hyaline to light olive in colour, septate at intervals, the septa being rather difficult to observe. The hyphae vary in width from 2–5 μ.

3. *Formation of spores.*

The "conidiophores" are very variable, and under certain conditions may be almost entirely absent. In all cultures the

Fig. 6. *Torula botryoides*, n.sp. *a*, conidiophore from culture in liquid fish extract, × 1500; *b*, conidiophore from culture on steamed potato showing method of spore-formation, × 2300.

spores are formed not only in dense heads on the conidiophores but also laterally in small groups of two or three along the ordinary hyphae. The "conidiophores" are never very distinct from the vegetative hyphae, and are usually much branched (Fig. 6). The ends of the branches of the conidiophores are more or less swollen, depending on the conditions of growth. On potato chunks at laboratory temperature the ends of the conidiophores form pronounced sterigmata, somewhat resembling those of *Penicillium*, and from which the conidia are abstricted, in long chains, as in *Penicillium*. On agar slopes at

2° C. the sterigmata are much less pronounced and the conidia appear to be borne in dense heads on the ends of the conidiophore. These apparent heads of conidia are made up of short chains, the base of each chain being attached to the conidiophore by a sterigma (Fig. 7).

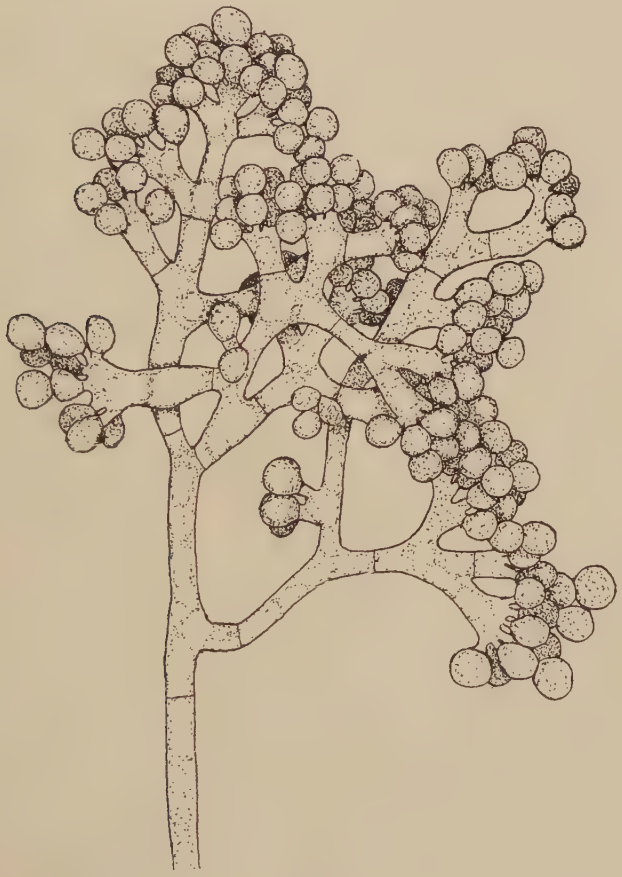


Fig. 7. *Torula botryoides*, n.sp. Conidiophore from culture on agar at 2° C. × 600.

4. *Spores.*

The spores are spherical to oval in shape, 4–8 μ × 4–6 μ in size, the mean size of the conidia being very constant under all conditions of growth. When young they are hyaline, but become olivaceous when older. The chains of conidia easily break up into separate spores. The walls are smooth.

II. Macroscopic Characters.

The fungus does not spore at all well on potato agar at ordinary temperatures. The growth is chiefly vegetative, dark olive in colour and almost entirely within the medium. On other solid media, e.g. steamed carrot or potato, growth is more profuse and greyish in colour, and the colonies develop masses of spores. The various isolations are uniform in appearance under cultural conditions, showing a great contrast to those of *Sporotrichum carnis* and *Cladosporium herbarum.*

III. Physiological Characters.

The various isolations of this fungus were grown at temperatures of − 6, 2, 12, 25, 30° C. No growth took place at 25° or 30° C. At the ordinary laboratory temperature growth was fair but resulted in the formation of few spores upon potato agar, the cultures consisting almost entirely of mycelium within the agar slants. At 2° C. a copious aerial growth was produced upon the same medium, giving the culture a greyish-white appearance, whereas at laboratory temperature, the fungus was dull brown or black in colour. The fungus formed an abundance of spores on the agar cultures at 2° C., these being produced in groups of short chains on much branched conidiophores, as well as in isolated groups along the hyphae. Growth occurred at − 6° C., but was very slow, even slower than in *Cladosporium herbarum.*

IV. Discussion.

The systematic position of this fungus is obscure. On fish and meat, from which it was originally isolated, it was a greyish-white, woolly mould. In cultures on potato agar the colonies are brownish at ordinary temperature. In view of this, the fungus is considered to belong to the Dematiaceae, a conclusion supported by the formation of conidia which are hyaline to olivaceous in colour. The branched conidiophores with their sterigmata bearing spores, seem to be peculiar to this fungus, but for the present it is included in the genus *Torula.* The general appearance of the young conidiophores suggests the specific name *botryoides.* The diagnosis is as follows:

Torula botryoides n.sp.*

Colonies grey-white to fuscous, woolly, ¼–1″ in diameter. *Hyphae* septate, hyaline to light olive, 2–5 μ wide.

* *Torula botryoides* sp.nov.
Coloniis cinereo-albis vel fuscis, lanosis, 0·7–2·5 cm. diam., hyphis septatis hyalinis vel pallide olivascentibus, 2–5 μ latis. Conidiophoris e mycelio haud diversis, valde ramosis; conidiis in catenulis e sterigmatibus vel in acervulis

Conidiophores not distinct from the vegetative mycelium, much branched.

Conidia produced basipetally in long chains from sterigmata, also in groups of 1–3 along hyphae, hyaline to olivaceous, 4–8 μ × 4–6 μ, spherical to oval. On potato agar at ordinary temperatures colonies brown-black, and partly sterile. On steamed potato or carrot the colonies are grey-white and spore freely.

Habitat on fish and meat kept in cold storage.

Kr. Høye (6) describes a species of *Torula, T. epizoa,* upon dried salted cod in Norway, causing brown spots on the fish. Experiments showed that its growth was much restricted if no salt were present in the medium. Its growth was best in fish extract containing 10 % of salt. The colonies found on dried fish consisted mainly of a dense layer of chains of spores abstricted from the mycelium. The present fungus, *T. botryoides,* shows none of the above characters. On meat in cold storage the fungus forms greyish-white, rather fluffy patches, and its growth in cultures is much restricted if salt is added to the medium. Høye describes a peculiar cellular stroma developed by his fungus when grown in cod-extract gelatine containing less than 10 % salt, hyphae being very feebly developed. No such mode of germination has been observed in *Torula botryoides* under any conditions. It is clear that the two species are distinct.

W. G. Farlow (5) describes a fungus *Oidium* (*Torula*) *pulvinatum,* also found on the surface of dried cod in America, causing brown spots, but his diagnosis is not applicable to *T. botryoides,* as he states that the spores of *T. pulvinatum* are only 3–5·5 μ in diameter, and 12–15 in a chain, whereas the spores of *T. botryoides* are rather larger, and the chains may be much longer or absent. His drawing of a young conidiophore bears no resemblance to that of *T. botryoides. Oidium* (*Torula*) *pulvinatum* is probably identical with *Torula epizoa* but the diagnosis of these forms is inadequate.

V. *WARDOMYCES ANOMALA* n.gen. and n.sp.

This fungus was isolated once during the course of the investigation from a white, slightly woolly patch of mould on one of a consignment of skinned Australian rabbits which had been condemned on arrival here in consequence of serious contamina-

1–3nis e hypharum lateribus ortis, hyalinis vel olivascentibus, 4–8 μ × 4–6 μ, sphaericis vel ovalibus. Coloniae in agaro Solani tuberosi temperie normali cultae brunneo-atrae et partim steriles, in Solano cocto et in Dauco cinereo-albae et libere sporiferae sunt.

Hab. Ad carnem in frigidariis asservatam.

tion by mould growths. On the rabbit, this particular mould was almost indistinguishable from *Sporotrichum carnis*, but upon isolation it showed quite different microscopic characters.

The spores germinate to form a branched mycelium of hyaline hyphae 2–4 μ wide. The conidiophores arise as short, lateral branches, 15–25 μ long, from the vegetative hyphae; they remain hyaline and become septate with age. Some conidiophores are unbranched and form rarely short chains of two or three conidia, the distal conidium being the oldest; others branch repeatedly and form heads of spores which usually arise separately, although under certain cultural conditions two spores are formed occasionally in a chain. Two or three spores are often produced on each of the terminal cells of the branched conidiophore, one spore being formed terminally and the others in succession laterally (Figs. 8–10).

Fig. 8. Conidiophore of *Wardomyces anomala*, n.sp., from culture on agar. × 1500.

Fig. 9. Conidiophore of *Wardomyces anomala* n.sp., showing short spore chains. × 2300.

a *b* *c*

Fig. 10. Spore formation of *Wardomyces anomala*, n.sp. (*a*) Stage 1, from drop-culture 3 days old, × 1500. (*b*) Stage 2, 24 hours after stage 1, × 1500. (*c*) Stage 3, 24 hours after stage 2, × 1500.

The conidia are dark brown to black in colour, and vary in shape from sub-spherical to oval, with slightly pointed ends. They are 5–8 μ × 4–6 μ in size, with smooth walls which are almost opaque. The fungus grows well on the usual artificial media at ordinary temperature, but although isolated from rabbits which had been kept in cold storage does not grow at temperatures just above freezing point.

This fungus appears to be the type of a new genus of the Dematiaceae, the characteristic feature being the successive lateral proliferations of the basal cells of the conidiophores. The genus is named *Wardomyces* in memory of the late Prof. Marshall Ward.

Wardomyces n.gen.*

Mycelium creeping, septate, hyaline. Conidiophores formed as lateral branches of the mycelium, short, branched, septate, hyaline, the branches arising as successive lateral proliferations of the basal cells. Conidia abstricted singly, arising from the terminal cells of the conidiophores in lateral succession in groups, oval to spherical, brown to black.

The species diagnosis is as follows:

Wardomyces anomala n.sp.†

Colonies circular, white to fuscous, adpressed to the substratum, $\frac{1}{8}$–$\frac{1}{4}$″ in diameter.

Hyphae creeping, branched, septate, 2–4 μ wide, hyaline.

Conidiophores short, 15–25 μ long, scarcely differentiated from the vegetative hyphae, branched or unbranched, hyaline.

Conidia abstricted singly, brown to black, smooth, subspherical to oval, usually with slightly pointed ends, 5–8 μ × 4–6 μ.

Habitat on flesh of rabbits kept in cold storage.

VI. *PENICILLIUM* spp.

Forms of the common blue-green mould were of frequent occurrence upon cold-store meat, and were occasionally found

* *Wardomyces* gen.nov.

Mycelio repente, septato, hyalino, conidiophoris e mycelio lateraliter exorientibus, brevibus, ramosis, septatis, hyalinis, ramis e cellulis basalibus successive emissis. Conidiis singillatim abstrictis, e cellulis conidiophorarum terminalibus successione laterali gregatim exorientibus, ovalibus vel sphaericis, e brunneo nigrescentibus.

† *Wardomyces anomala* sp.nov.

Coloniis circularibus, e albis fuscescentibus, substrato adpressis, 3–6 mm. diam., hyphis repentibus, ramosis, septatis, 2–4 μ latis, hyalinis. Conidiophoris brevibus, 15–25 μ longis, ab hyphis vegetativis vix differentibus, ramosis vel simplicibus, hyalinis. Conidio singillatim abstrictis, e brunneo nigrescentibus, laevibus, subsphaericis vel ovalibus, plerumque utrinque subacutis, 5–8 μ × 4–6 μ.

Hab. Ad cuniculorum carnem in frigidariis asservatam.

upon bacon also. When young, the colonies are white in colour, but when mature they are bluish green. The isolations proved generally to be *P. expansum*, but one has been provisionally identified by Dr Thom of Washington as *P. asperulum* Bainier.

Some evidence has been obtained that certain isolations of *P. expansum* from cold-store meat will germinate and grow at − 6° C., but development is very slow and after more than two years the colonies in the culture tubes or on meat are little more than just visible to the naked eye. Growth at this low temperature is sometimes more marked if germination of the spores has proceeded for 24–48 hours before the culture tubes are placed in the cold store. At − 1° to − 0·5° C. growth is more vigorous and at 2° C. it is active.

The spores and young mycelial growths also of this fungus are able to withstand exposure to a temperature of − 6° C. for long periods. Growth has sometimes taken place or has been resumed after 2½ years at this temperature.

The large colonies of *Penicillium* seen on contaminated meat are in marked contrast to the small growths which have been produced under experimental cold storage conditions, and point to the fact that at some time or other the temperature of the meat has risen to about or just above freezing point. Spores of *Penicillium* are always present in the air, and doubtless are deposited upon the surface of the meat, ready to develop if conditions are suitable for growth. These bluish-green moulds are entirely superficial.

VII. *SACCHAROMYCES* spp.

Both white and pink yeasts were of common occurrence on meat contaminated with mould fungi. These forms develop with great rapidity at temperatures just above freezing point, but there is no evidence yet that they will grow below zero. The fungus described some years ago by Klein (10) as the cause of brown spots on chilled beef should, perhaps, be placed here. Yeast colonies in a dry condition are often brownish in colour, but it is not possible to be certain of the identity of the organism which Klein described. The white and pink yeasts isolated from meat do not form spores, and hence belong to the genus *Torula* used in the sense of Hansen and Jörgensen.

VIII. *THAMNIDIUM* spp.

Species of *Thamnidium* were frequently isolated from meat of various kinds in cold stores, which had become contaminated by mould growths, the type of which known in the meat trade as "whiskers" being usually due to species of this genus. Upon

meat these growths are profuse and are practically indistinguishable from species of *Mucor*. The genus *Thamnidium* differs from *Mucor* in the presence of two different kinds of sporangia, large and small, but in the natural occurrence of *Thamnidium* upon meat the small sporangia are either few or non-existent so that it appears like a *Mucor*. When grown under laboratory conditions, however, both types of sporangia are usually formed; upon nutrient agar small sporangia predominate, but upon cooked meat the reverse is the case. Upon fresh meat in the laboratory large sporangia sometimes occur to the exclusion of small ones.

Two species of *Thamnidium* were frequently isolated from cold-store meat, *T. elegans* and *T. chaetocladioides*, and of these the latter was the more common. It is of interest that these fungi are of rare occurrence upon other substrata, although upon mouldy meat they seem to be particularly common.

At ordinary temperatures and up to 20° C. these species grow well; at 25° C. *T. elegans* grows fairly well but *T. chaetocladioides* develops hardly at all, the spores becoming much swollen and forming curious amoeboid-looking cells. At 30° C. neither species develops.

So far there has been only the slightest signs of growth of either of these species at − 6° C., but at a temperature of 1–2° C. they grow profusely. They also grow at a temperature of − 1° to − 0·5° C.; development at temperatures between this and − 6° C. has not yet been tested. Bidault (2), however, states that *T. chaetocladioides* (= *Chaetostylum Fresenii*) grows at − 10° C.

The spores and even young mycelia will retain their vitality for long periods at a temperature of − 6° C. Thus meat inoculated with spores of *Thamnidium chaetocladioides* in October 1919 and placed in the cold store either immediately or after 24 hours, developed profuse growths of this fungus directly after removal to ordinary temperature in January 1921.

Mould spores of this type, therefore, which may have been deposited on the meat before being placed in store, remain living for long periods at low temperatures, and if there is a breakdown of the refrigerating plant, causing a rise in temperature to about freezing point, it is to be expected that these lurking moulds will develop profusely. Where such "whisker" growths are apparent on the meat, it is probable that the meat has been exposed to a temperature of 0° C. or slightly above for some time during storage. These moulds are entirely superficial and can readily be removed with a cloth. If unaccompanied by putrefactive bacteria, meat affected by these moulds is not dangerous for human consumption.

IX. *MUCOR* spp.

It was practically impossible to distinguish mould growths belonging to this genus from those of *Thamnidium* until the forms were isolated in culture. Together with *Thamnidium*, this genus is responsible for the profuse, greyish-white growths upon cold-store meat known as "whiskers," but species of *Mucor* are less frequently met with in this connection than are *Thamnidium elegans* and *T. chaetocladioides*.

Three species of *Mucor* were isolated from contaminated beef and mutton and these have been kindly identified by Prof. Lendner of Geneva as

M. mucedo Linné,
M. lusitanicus Bonderlein, and
M. racemosus Fres.

M. mucedo and *M. racemosus* are common moulds occurring upon a great variety of substrata.

Like *Thamnidium*, these fungi grow well at 1–2° C., but apart from slight germination of the spores of *M. mucedo* at – 6° C. there has been no indication of growth at this temperature. Tests have not yet been carried out between – 6° and 0° C. At temperatures just above zero, chlamydospores are formed more profusely than at ordinary temperatures.

M. mucedo and *M. lusitanicus* grew well and produced sporangia at all temperatures between 2° C. and 25° C., but did not grow at 30° C. *M. racemosus* grew at 30° C., but no sporangia were formed at that temperature.

The behaviour of these species of *Mucor* on cold-store meat is similar to that of *Thamnidium* and the presence of profuse growths of these forms is probably to be correlated with a rise in temperature to just above freezing point. Like *Thamnidium*, these species of *Mucor* do not penetrate the meat to any extent, nor do they confer poisonous properties upon it.

X. SUMMARY.

(1) The fungi which occur on cold-store meat coming to England from the southern hemisphere have been systematically examined. These moulds are: *Cladosporium herbarum* (the cause of meat "Black Spot"), *Thamnidium chaetocladioides*, *Thamnidium elegans*, *Mucor racemosus*, *Mucor mucedo*, *Mucor lusitanicus*, *Penicillium expansum*, *Penicillium anomalum*, *Saccharomyces* spp. together with two new species, *Sporotrichum carnis* and *Torula botryoides*, and the type species of a new genus, *Wardomyces anomala*.

(2) A general survey of many forms of *Cladosporium* has been undertaken, with the result that many so-called species of *Cladosporium* including *C. epiphyllum* are interpreted as strains

of *C. herbarum* and not distinct species. *Hormodendron cladosporioides* is a spore form of *C. herbarum*, and under cultural conditions is produced at low as well as at high temperatures.

(3) Some strains of *Cladosporium herbarum* will develop from spores at a temperature of − 6° C. and will give rise to considerable growths including conidiophores under prolonged cold-storage conditions. *Torula botryoides*, *Sporotrichum carnis*, *Penicillium expansum* and *Thamnidium* spp. sometimes develop slightly at this temperature, but readily at 0° C., and it is probable that they grow appreciably between these two temperatures; profuse growths of these forms on meat are usually an indication that the temperature has been raised to 0° C. or slightly higher at some time or other during storage. *Mucor* spp., *Saccharomyces* spp., and *Wardomyces anomala* do not develop at − 6° C., but will grow at 0° C. or just above.

(4) Spores and young mycelia of certain of these moulds, notably *Thamnidium* spp. and *Penicillium expansum*, retain their vitality for more than two years at − 6° C., and germinate or continue to develop on removal to ordinary temperatures.

(5) The growth of these moulds on meat is superficial, and even in "Black Spot" the mycelium penetrates only to a maximum depth of 4 mm. These fungi do not confer poisonous properties on the meat, and, unless associated with putrefactive bacteria, do not render the meat unfit for food.

(6) Several of these moulds are of common occurrence on vegetable debris and animal excreta, and their source is substrata of this nature occurring in and around abattoirs in the southern hemisphere. Air-borne spores alight upon the carcases before and during storage, and develop into mould growths at favourable opportunities.

(7) By controlling the temperature and humidity conditions in the cold stores, and by avoiding unduly prolonged storage, the growth of these fungi can be prevented.

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(14) Rabenhorst, L.—Kryptogamen-Flora.
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(17) Tabor, C. J.—Black spot on chilled and frozen meat. Cold Storage and Produce Review, Oct. 20th, 1921.
(18) Talayract, M. J.—Conserves de viandes par les procédés frigorifiques. Ann. d'hygiene publique, sér. 3, XLV, p. 166 (1901).

OBSERVATIONS AND EXPERIMENTS ON CEREAL RUSTS IN THE NEIGHBOURHOOD OF CAMBRIDGE, WITH SPECIAL REFERENCE TO THEIR ANNUAL RECURRENCE*.

By Karm Chand Mehta, M.Sc.
(*Professor of Botany, Agra College, Agra, India.*)

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PART I.

INCIDENCE AND MEANS OF OVERWINTERING.

1. Introduction.

The annual origin of rusts on cereals has been the subject of numerous investigations. In recent years several valuable contributions have been made on different aspects of the problem, attaching special importance to one factor or the other as the cause of infection.

The chief sources of "primary" infection, as has been pointed out, might be (i) uredospores that may survive on the cereals through the critical periods, (ii) an inherited germ of disease within the seed grain, as suggested by Eriksson*, (iii) sori within the seed grain, (iv) aecidiospores on some alternate host, (v) sporidia arising from teleutospores, (vi) uredospores living during the critical period on other grasses.

As far as England is concerned, one finds that no special study of the problem has been made. In literature one comes across only a few general statements based mostly on observation of the fact that the uredo-stage of some rusts can be found every now and then on cereals or wild grasses even during winter and the earlier part of spring—Plowright†, Ward‡ and Grove§. It was desirable therefore that a detailed investigation of the problem should be undertaken at an important wheat growing area in this country.

The work was commenced at the suggestion of Mr F. T. Brooks in October 1920 and carried out for nearly two years at the Cambridge Botany School.

In this part of the paper I propose to give a brief account of the incidence of rusts on wheat in the vicinity of Cambridge and also to discuss the relative importance of the various factors which go to explain the origin of rust outbreaks year after year.

Throughout the period under report, field observations were made as far as possible once a week at the Cambridge University farm and occasionally also at other farms in the vicinity. In addition to the rusts of wheat, observations were regularly made on the black rust of Couch grass (*Agropyron repens*). During the latter half of the period casual observations were also made on the rusts of barley and rye.

Side by side with field work, extensive culture work was

* Carleton, M. A., U.S. Dept. Agric. Div. Veg. Phys. and Path. Bull. No. 16 (1899); Eriksson, J., Compt. Rend. CXXIV, p. 475 (1897).

† Plowright, C. B., Gard. Chron., N.S. XVIII, p. 234 (1882).

‡ Ward, H. M., Ann. Bot., XIX, p. 1 (1905).

§ Grove, W. B., British Rust Fungi (1913).

carried on for a period of nearly two years, with the object of throwing further light on some of the more important physiological differences between the yellow, the brown, and the black rusts of wheat.

2. Review of the Literature.

It is unnecessary to enter into a historical survey of the work done on the cereal rust problem, in view of the fact that it has so often been summarised. In referring to comparatively recent work in different countries, my object is to bring out the various views expressed on the significance of the more important factors that fall within the scope of this paper.

1. Dealing first with the possibility of the uredospores occurring during the critical periods on self-sown plants being the source of infection, one may refer to the observations made by McAlpine* in Australia. In that country *Puccinia graminis* is perpetuated from season to season by means of uredospores. It is important in this connection to note that in Australia it is the heat and drought of summer, and not the winter cold, against which the fungus has to provide.

In some parts of the United States of America, Carleton†, Hitchcock and Carleton‡ and Bolley§ have remarked that fresh uredospores of *Puccinia Rubigovera* are found throughout winter. In a later contribution Bolley‖ has stated that the uredospores of *P. graminis* also can withstand the cold of a North Dakota winter. Carleton†, however has denied the possibility of the black rust (*P. graminis*) of wheat being able to overwinter in Kansas. Again Pritchard¶ has pointed out that the uredospores of *P. graminis* when kept in the open lost their viability in two months' time. The same author also remarked that in North Dakota the wintering of *P. graminis* as mycelium in plant tissues is very doubtful.

Hoerner** has made a similar observation about the brown rust of oats.

Jaczewski†† has stated that black rust in Russia cannot overwinter and that even the mycelium of this rust inside the host cannot withstand frost.

* McAlpine, D., The Rusts of Australia (1906).
† Carleton, M. A., U.S. Dept. Agric. Div. Veg. Phys. and Path. Bull. No. 16 (1899).
‡ Hitchcock, A. S. and Carleton, M. A., Kansas Agric. Coll. Exp. Stat., Bull. 46 (1894).
§ Bolley, H. L., Centralbl. f. Bakt. IV, Abt. 2, p. 893 (1898).
‖ Bolley, H. L., Science, XXII, p. 50 (1905).
¶ Pritchard, F. J., Bot. Gaz. LII, p. 169 (1911).
** Hoerner, G. R., Amer. Journ. Bot. VIII, p. 452 (1921).
†† Jaczewski, A., Zeitschrift für Pflanzenkrankheiten, XX, p. 321 (1910).

Klebahn* has remarked that in Germany *P. dispersa* and *P. glumarum* can overwinter in the uredo-stage, but *P. graminis* cannot do so.

On the other hand Eriksson and Henning† state that in Sweden none of the three rusts under report can overwinter in the uredo-stage.

In India Butler‡ has stated that self-sown plants are very rare and probably quite absent in the hotter parts of that country, where, like Australia, the period most critical for rusts is the intensely hot summer. He has further remarked that attempts to preserve uredospores in the earlier part of the hot weather have been unsuccessful.

From a cursory perusal of the observations quoted above it is clear that on account of great climatic differences between the countries referred to, the incidence of rusts could not possibly have been similar. Apart from its incidence one has to enquire whether a certain rust can or cannot pass the unfavourable period inside the host in the mycelial stage. Again it is doubtful if the small number of uredospores that may have survived through the critical period would be enough to bring about a "spontaneous outbreak" of a certain rust. This question becomes still more difficult to answer in view of the fact, which will be proved later, that in a cold country rusts take a much longer time to develop pustules even during spring than has hitherto been believed.

2. Eriksson§, as is well known, has attributed the fresh outbreak of rusts to an internal, inherited and invisible germ of disease inside the seed grain which he considers may take from two weeks to nine months from the time of sowing to assume a visible form, but there are few believers in this "mycoplasm hypothesis" at the present day.

Bolley‖ has stated in contradiction to Eriksson's view that there is much evidence that all infection of new grain plants comes from without, and that there is nothing to prove an internal symbiotic life. Similarly the negative results obtained by Marshall Ward¶ in his pure culture experiments with seeds from rusted grasses, and those conducted by Butler and Hayman** at Cawnpore, prove conclusively that no rust develops from the "mycoplasm" suspected of being inside the seed grain.

* Klebahn, H., Die Wirtswechselnden Rostpilze (1904).
† Eriksson, J. and Henning, E., Die Getreideroste (1896).
‡ Butler, E. J., Fungi and Disease in Plants (1918).
§ Eriksson, J., Compt. Rend. CXXIV, p. 475 (1897); Ann. Bot. XIX, p. 55 (1905).
‖ Bolley, H. L., Proc. Amer. Assoc. Adv. Science, XLVII, p. 408 (1898).
¶ Ward, H. M., Ann. Bot. XIX, p. 1 (1905); Proc. Roy. Soc. LXIX, p. 451 (1902).
** Butler, E. J. and Hayman, J. M., Mem. Dept. Agric. India, Bot. Ser. 1, No. 2 (1906).

Again Bailey* has quite recently denied the possibility of a "primary" outbreak of the Hollyhock rust from internal infection.

In the absence of a positive proof of the origin of rust on seedlings grown under conditions that would forbid all external infection, we cannot but conclude that, although Eriksson's researches on cereal rusts have been exceedingly useful, his suggestion about their propagation through "mycoplasmic symbiosis" does not carry us any nearer to the solution of the problem of their recurrence.

3. With reference to the view that infection is carried from year to year by means of infected seed, it is interesting to note that Pritchard† has sketched definite figures of the mycelium of *P. graminis* inside seedlings from grain that had teleuto-sori facing the embryo. He has however not been able to record the appearance of any pustules on such plants. Beauverie‡ also believes in the transmission of rust through infected seed. Recently Hungerford§, on the strength of elaborate experiments, has strongly denied the possibility of the propagation of rust from one crop to the next by means of rusted seed grain, and so has Eriksson‖.

4. While discussing the importance of intermediate hosts, one has to bear in mind the fact that as far as yellow rust is concerned, no such host has yet been discovered. As regards the brown rust of wheat (*P. triticina*), only recently Jackson and Mains¶ have been able to achieve successful infection of species of *Thalictrum* with its sporidia.

In the case of black rust too McAlpine** states that in Australia the barberry plays no part as an intermediate host, the rust being perpetuated from season to season by uredospores. Henning†† has also expressed the same view about warm countries in general where the barberry does not exist.

Butler‡‡ has stated that aecidiospores found on barberry in some of the hills could not infect wheat; and has concluded therefore that the aecidial stage is of no account in India.

Pritchard§§ has remarked that the absence of barberry, or its

* Bailey, M. A., Ann. Bot. xxxiv, p. 173 (1920).
† Pritchard, F. J., Phytopathology, i, p. 150 (1911).
‡ Beauverie, J., Rev. Gén. Bot. xxv *bis*, p. 11 (1914).
§ Hungerford, C. W., Journ. Agr. Res. xix, p. 257 (1920).
‖ Eriksson, J., Phytopathology, xi, p. 385 (1921).
¶ Jackson, H. S. and Mains, E. B., Phytopathology, xi, Abstracts, p. 40 (1921).
** McAlpine, D., The Rusts of Australia (1906).
†† Henning, E., Tidsk. f. Landtmän, xxviii, 1917. Abstract in Hedwigia, lxi, p. (49) (1919).
‡‡ Butler, E. J., Fungi and Disease in Plants (1918).
§§ Pritchard, F. J., Bot. Gaz. lii, p. 169 (1911).

existence at very distant places, makes spring infection of wheat inexplicable, and has suggested the possibility of infection of wheat due to teleutospores being of greater importance.

Lastly Eriksson* inclines also to the view that propagation to and from the aecidial host is very slight.

On the other hand one learns from the contributions of Plowright†, Stakman and Piemeisel‡, Freeman and Johnson§, Lind‖, Güssow¶, Broadbent** and others that there is a definite connection between the aecidium on barberry and the distribution of black rust on graminaceous hosts. To avoid repetition, this factor and the next two will be discussed in detail later in the text.

The present work was taken up chiefly with the object of finding out (i) if one could satisfactorily explain the fresh attacks of different rusts on wheat in spring, taking the uredospores surviving on self-sown plants during winter as the source of infection; (ii) whether the amount of such uredospores could, with a reasonable amount of certainty, be held responsible for the more or less spontaneous outbreak of rusts over large areas of winter wheat; (iii) if one could find the cause of the more or less fixed sequence in the appearance of the three rusts; and also (iv) the relative importance of some of the other factors already referred to.

3. Black Rust (*Puccinia graminis* Pers.).

(*a*) *Incidence.*

This rust does not appear on graminaceous hosts until late in the season. In the year 1921 it was first noticed towards the end of June on Couch grass (*Agropyron repens*). In the vicinity of Cambridge it is not common, and it has not been found on any other local wild grass. Amongst the cereals it was observed on wheat alone in the year 1920 and only on barley last year (1921).

(1920–21.) There was plenty of the uredo-stage on wheat (self-sown) and Couch grass in October but later on no fresh uredo-sori were observed. In January 1921 one could find only old and disfigured uredo-sori. No black rust was observed at

* Eriksson, J., Bot. Gaz. xxv, p. 26 (1898).
† Plowright, C. B., Gard. Chron., N.S. xviii, p. 234 (1882).
‡ Stakman, E. C. and Piemeisel, F. J., Journ. Agr. Res. x, p. 429 (1917).
§ Freeman, E. M. and Johnson, E. C., U.S. Dept. Agr. Bur. Plant. Indus. Bull, p. 216 (1911).
‖ Lind, J., Danish Fungi as represented in the herbarium of E. Rostrup (1913).
¶ Güssow, H. T., Phytopathology, iii, p. 178 (1913).
** Broadbent, W. H., Journ. Minis. Agric. xxviii, p. 117 (1921).

the University farm on self-sown plants or regular crops in the spring or summer of 1921.

(1921–22.) No black rust was found on cereals or wild grasses at the University farm in the autumn of 1921 or the spring or summer of 1922, even up to harvest time.

It may be pointed out that the greater portion of the plot under observation in the autumn of 1920 was left unploughed and one could see a large amount of old straw with teleutosori throughout the spring and summer of 1921. The new crop of wheat was sown on the adjacent plot.

This total absence of black rust goes a long way to disprove the possibility of direct infection of graminaceous hosts by sporidia produced on the germination of teleutospores which were present in plenty on the adjacent plot.

It is essential in this connection to state that there are no plants of wild barberry known to exist in the near neighbourhood of the University farm.

(*b*) *Overwintering of the uredospores.*

It is very doubtful if fresh pustules of black rust are at all developed during the winter months in this locality. During the last two winters (1920–21, 1921–22), although comparatively mild, no such pustules were observed. Moreover the complete absence of this rust on self-sown plants of wheat which bore uredo-pustules of both yellow and brown rusts during the greater part of the cold season (1920–21) indicates that black rust cannot stand the cold of an English winter. Similarly Couch grass (perennial as it is), which was once covered with this rust in the autumn of 1920, has never shown a pustule of it at the University farm since that time.

Plants of rusted Couch grass transplanted to pots in November and kept in the open showed no uredo-sori after two months and have since then been quite free from them.

As regards the viability of the uredospores collected from the open in winter, it may be pointed out that inoculations tried on wheat in the laboratory during November and early in December 1920 gave satisfactory results and the spores showed good germination. By the second week of December the spores had practically lost their viability, and consequently it was impossible to infect seedlings even in the laboratory with uredospores (old as they were) from the open after that date.

That the loss of viability was due to the effect of cold is clear from the fact that since November 1920 up to July 1922 the writer successfully kept a culture of this rust in the laboratory.

(*c*) *Hibernation through sori in seed.*

Reference has already been made to the experiments conducted by Hungerford* with rusted grain of wheat. In the summer of 1921 while carrying on some work on the specialization of black rust, some seedlings of Couch grass were raised from healthy as well as from badly rusted grain collected in the autumn of 1920. It was found that rusted grain invariably gave healthy plants, and there was no trace of rust even on plants as many as ten weeks old. Only those seedlings which were artificially inoculated developed the disease, and the controls never became rusted. The writer is in complete agreement with the view expressed by Hungerford, that propagation of rust through infected seed is improbable.

(*d*) *Aecidium on barberry and fresh outbreaks of rust.*

Recently Stakman and Piemeisel† have stated that they are in doubt as to the importance of the overwintering of the uredostage on grasses, but have remarked that barberry may be important at least locally.

Similarly Freeman and Johnson‡ have observed that aecidiospores may be sufficient in number (in spite of their short viability) to cause epidemics on grasses.

Broadbent§ has pointed out that there is a plentiful distribution of the common barberry in the counties of Carmarthenshire, Cardiganshire and Pembrokeshire, and that black rust on wheat is generally distributed over the same area. In all other parts of the British Isles he states that black rust is scarce.

Lastly Lind‖ and Güssow¶ have made an important statement that the gradual extermination of barberry since 1903 has brought about a great decrease in the severity of black rust in Denmark.

The complete absence of black rust in the summer of 1921 at the University farm has been noted above but it was found on Couch grass and barley at a farm near Cambridge in the same season. This farm was known to have a few plants of barberry in the hedgerow which were kept under observation from the beginning of spring. Ripe aecidia were noticed on the barberry on the 6th of June and till then there was no trace of black rust although the standing crop of barley had plenty of *P. simplex*

* Hungerford, C. W., Journ. Agr. Res. XIX, p. 257 (1920).

† Stakman, E. C. and Piemeisel, F. J., Journ. Agr. Res. X, p. 429 (1917).

‡ Freeman, E. M. and Johnson, E. C., U.S. Dept. Agr. Bur. Plant. Indus. Bull. p. 216 (1911).

§ Broadbent, W. H., Journ. Minis. Agric. XXVIII, p. 117 (1921).

‖ Lind, J., Danish Fungi as represented in the herbarium of E. Rostrup (1913).

¶ Güssow, H. T., Phytopathology, III, p. 178 (1913).

and also yellow rust here and there. On the 24th of June for the first time in the season Couch grass just below infected bushes of barberry was seen covered with uredo-sori of black rust. By the middle of August the disease had spread to barley as well. The attack was a mild one and rusted plants were observed only within ten to fifteen yards of the hedgerow. By the third week of October the uredo-stage became very rare and during winter and the following spring (1922) no uredo-stage could be found.

Towards the end of May ripe aecidia on barberry were again noticed at the same farm. A fortnight later the uredo-stage was quite common on Couch grass just below the infected bushes of barberry. By the end of July the rust had spread on Couch grass over a considerable area, the weather being very favourable.

One could safely attribute the exceedingly restricted and mild nature of the attack of black rust as recorded above and its complete absence at the University farm, last year (1921), to the exceptionally dry and warm weather during the latter part of spring and the whole of summer. Undoubtedly the weather conditions were most unfavourable, both for an extensive infection by the aecidiospores and also for the spread of this rust by the uredospores. This probably explains why black rust was conspicuous by its absence last summer at the University farm. It may be pointed out that last summer wheat and barley were simply covered with yellow and brown rusts which it must be remembered had already established themselves during the earlier part of the year.

(*e*) *Inoculation experiments with sporidia.*

Eriksson and Henning* while denying the possibility of the survival of uredospores through the winter have attached special importance to the view that teleutospores may on germination cause direct infection of graminaceous hosts by their sporidia.

In the spring of 1921 some inoculation experiments were conducted on young seedlings of wheat to see if they could be infected with sporidia of *P. graminis tritici*.

From the 22nd of February up to the 6th of April several germination tests were made with teleutospores that had been kept in the open throughout winter, and they invariably showed good germination. On the 6th of April two plants of barberry (as control) and many young seedlings (six days after sowing) of wheat were inoculated with teleutospores mixed in water. On the fourteenth day inoculated leaves of barberry showed spermogonial patches. Seedlings of wheat showed only brownish

* Eriksson, J. and Henning, E., Die Getreideroste (1896).

streaks on the stem. These streaks were fixed and microtomed but no mycelium could be discovered. No pustules of rust were noted on wheat even up to the 20th of May (nearly six weeks after inoculation).

These negative results prove that sporidia cannot infect the graminaceous host directly.

(*f*) *Inoculations with aecidiospores.*

That the latter part of spring (1921) was unfavourable for an extensive infection of cereals by aecidiospores from barberry in this locality will be clear from the following observations.

During the months of June and July as many as six attempts were made to inoculate seedlings of wheat and Couch grass with aecidiospores from barberry in the open, or with such material as had been cultivated in the laboratory. All the inoculations were made in a cool green-house.

Excepting once, the aecidiospores from the open showed poor germination and out of thirty-six seedlings of Couch grass only three took infection. In the case of wheat (Red Sudan) out of forty-one seedlings only seven developed uredo-sori. The pustules on wheat it must be pointed out were exceedingly small (very much smaller than those on Couch grass). This form was later on found to be *P. graminis secalis*.

In summing up one may state that as far as the neighbourhood of Cambridge is concerned, and the same is probably true of other parts of England, the appearance of black rust year after year starts with the fresh infection of cereals by the aecidiospores from barberry. Also that there is no possibility of the perpetuation of this rust through overwintering uredospores, even after comparatively mild winters, like the two under report.

The question of the possibility of the survival during winter by means of the mycelium inside the tissues of the host plant, will be discussed later on.

4. The Brown Rust (*P. triticina* Erikss.).

(*a*) *Incidence.*

A study of the incidence of this rust, and its culture in the laboratory is interesting, as it combines the power of resistance to cold, so characteristic of yellow rust, with the capacity to withstand warmer weather, as exhibited by black rust. The rust is quite abundant in the neighbourhood of Cambridge and, as Grove* has mentioned, in England as a whole. It appears on

* Grove, W. B., British Rust Fungi (1913).

wheat early in spring usually a little later than the yellow but long before the black rust. Butler* has stated that in India it is the earliest to appear on wheat. The uredo-sori of this rust in summer resemble those of black rust on leaves and are often mistaken for them.

Ward†, Carleton‡, Hitchcock and Carleton§, Bolley‖ and others to whom reference has already been made have pointed out that the uredospores of brown rust can be found during winter. The following is a brief summary of the observations made by the writer.

(1920–21.) There was plenty of the uredo-stage of the rust on self-sown wheat in October and November but after a heavy frost in December it was very rare for a period of two months though not altogether absent. By the third week of February it again became quite common on self-sown wheat. For the rest of the season up to the harvest time the rust was plentiful on the wheat crops. Barley was observed to be heavily infected by *P. simplex* and the brown rust of rye too was very common.

(1921–22.) After the harvest of 1921 it was observed here and there round Cambridge and the brown rusts of barley and rye were also noted on self-sown plants. During autumn and the whole of winter the rust was not difficult to find on wheat except for a short time in January 1922 when it was rather rare. *P. simplex* was also noted on barley occasionally. By the middle of March it had established itself on the crop and was very common in summer (1922) up to the harvest time.

In addition to the above may be quoted some interesting observations, made on a small plot of Luther Burbank wheat (a very susceptible variety) sown at the Botanic garden on August 10th, 1921. This plot was kept under frequent observation. Throughout the season (1921–22) brown rust was found on this plot. Fresh uredo-sori were, however, rather rare in the latter half of January after frost. It is of interest to note that this plot showed no yellow rust for a period of over ten months.

As far as this rust is concerned, it may safely be stated that for the period the data cover it is transmitted from season to season by uredospores.

We cannot, however, take the last two winters as anything

* Butler, E. J., Fungi and Disease in Plants (1918).
† Ward, H. M., Ann. Bot. XIX, p. 1 (1905).
‡ Carleton, M. A., U.S. Dept. Agric. Div. Veg. Phys. and Path. Bull. No. 16 1899).
§ Hitchcock, A. S. and Carleton, M. A., Kansas Agric. Coll. Exp. Stat., Bull. 46 (1894).
‖ Bolley, H. L., Centralbl. f. Bakt. IV, Abt. 2, p. 893 (1898).

like a severe test; moreover after a frost or during a cold spell this rust too was very rare though not altogether absent. For these reasons it is doubtful, if one could safely attribute the outbreaks on the autumn-sown crop early in spring to the few uredospores that may be lurking in the open. This rust may be altogether absent during the greater part of a severe winter. Eriksson and Henning* have observed such a condition in Sweden.

At the same time one cannot conclude, from the absence of uredo-sori even for the whole period during a severe winter, that uredospores from the last season's crop have no significance as a source of infection to the autumn-sown (new) crops. The real source of danger is the abundance of uredospores on self-sown plants at autumn time and they will have done their work before the winter sets in.

(*b*) *Overwintering of the uredospores.*

As regards the viability of the uredospores of this rust taken from the open during winter, the writer agrees with Plowright† who found that spores which had been exposed to several nights of frost germinated with the greatest freedom. Further it may be added that during the last two seasons several inoculations on wheat were made in the open during winter with fresh uredospores of this rust resulting in satisfactory infections of the host.

(*c*) *Aecidium on* Thalictrum.

It is difficult to say anything definite on the possible infection of wheat by the aecidiospores found on species of *Thalictrum*. The aecidial stage on *T. flavum* in this country has been connected with *P. persistens* on *Agropyron repens*. It is interesting, however, to note that the brown rust of wheat was observed in plenty in the latter half of February (1921) and this year it was noted on the wheat crop in the middle of March, whereas the aecidium on *T. flavum* occurs from May to July (Grove‡). Even if the aecidium on *Thalictrum* proves to be connected with *P. triticina*, it is unlikely to have anything to do with the fresh origin of that rust each year.

* Eriksson, J. and Henning, E., Die Getreideroste (1896).
† Plowright, C. B., Gard. Chron., N.S. XVIII, p. 234 (1882).
‡ Grove, W. B., British Rust Fungi (1913).

5. The yellow rust (*Puccinia glumarum* Erikss. and Henn.).

(*a*) *Incidence.*

This rust is usually the earliest to appear and was noticed in the year 1921 as early as the third week of January on winter wheat at the University farm. It can be easily distinguished from either of the other two rusts by the orange-yellow colour of its uredo-sori, which are always arranged in long rows.

As regards its incidence, there is absolutely no similarity between the data recorded for the two seasons under report as the following account will show.

(1920–21.) The uredo-stage was very common in October on self-sown wheat on a small plot just outside the cages at the University farm. The plot was ploughed early in November but there was plenty of the rust on self-sown plants on some of the beds inside the cages also. During December and the earlier part of January 1921 it was very rare and was found only as old pustules on withered leaves. On the 20th of January strips of fresh pustules were noted on several leaves of the winter wheat crop in cages sown in October 5–7th 1920. Since then the rust was seen spreading rapidly and took an epidemic form by the middle of March. Towards the latter half of July when the weather was exceedingly warm fresh pustules were rather rare. The harvest was reaped by the end of July.

(1921–22.) In the month of August the rust was very rare on self-sown wheat or barley. During September and October too it was practically absent and no trace was found of it round Cambridge (in spite of a very thorough and daily search) except four or five self-sown plants on the 25th of October. It may be pointed out that during this period the brown rusts of wheat and rye (*P. dispersa*) and the dwarf rust of barley (*P. simplex*) were fairly common. From the 25th of October up to the end of May (1922), a period of over seven months, no trace of this rust was seen in this locality. It was first noticed in the beginning of June, but in spite of very favourable weather up to the harvest time the attack was an exceptionally mild one.

(*b*) *Discussion of "Mycoplasm hypothesis."*

At this stage the writer may refer to other observations which were made on some of the most susceptible varieties of wheat and barley sown in the open last autumn (1921).

1. Seedlings of Red Sudan wheat raised in the laboratory were transplanted in a small plot outside the cages on August 24th. Some of the seedlings were transplanted to a bed outside the laboratory.

2. Seedlings of a Mesopotamian variety of barley, exceedingly susceptible to yellow rust, were transplanted outside the cages and also outside the laboratory.

3. Several self-sown plants of barley and wheat were transplanted on October 17th from the cages to the beds outside. There was plenty of mildew, but no rust on these plants.

All three sets of plants were frequently examined for the appearance of yellow rust under conditions most unfavourable for an infection from without, because of the almost complete absence, as far as could be seen, of yellow rust around Cambridge.

Up to the end of May 1922 no trace of yellow rust was observed on any of these plants. Occasionally a few pustules of *P. triticina* on wheat and *P. simplex* on barley were noticed on plants transplanted outside the cages. Brown rusts both on barley and wheat, as has already been pointed out, were present on self-sown plants from the previous harvest (1921). Again, as remarked above, while dealing with the incidence of brown rust, Luther Burbank wheat growing at the Botanic garden all along (Sept. 1921 to July 1922) showed uredo-sori of that rust, but not even once was yellow rust noticed on that plot up to the end of June 1922 (a period of over ten months).

It is difficult to understand why yellow rust should not have appeared on the winter crops, on the wheat at the Botanic garden, and even on plants of varieties known to be exceedingly susceptible, if there was anything like an internal germ of disease in existence. It is essential in this connection to point out that the variety of Mesopotamian barley referred to above was very badly smitten with yellow rust during the summer of 1921.

Plants of Red Sudan wheat and barley had been exposed in the open at the University farm for more than ten months, without showing a pustule of yellow rust.

To make this point still more forcible, it may be mentioned that wheat artificially inoculated with yellow rust on Oct. 26th, 1921, bore pustules in less than three weeks' time, so there could be little doubt about the weather being favourable for the internal germ of disease to manifest itself if it did exist.

Again wheat inoculated with the same rust early in March and kept in the open developed pustules in a fortnight.

The fact that brown rust was flourishing during the greater part of this period, and was never altogether absent, shows that climatic conditions were not unfavourable. Keeping the data of the incidence of this rust for the season 1920–21 and 1921–22 in mind, one cannot help suspecting, that there is a clear connection between the incidence about autumn time and the origin of rust on winter crops in the following spring, as the following chart will show.

Date	1920–21	1921–22
October	Common	Only 4 to 5 plants
November	Very common	Oct. 25th. None afterwards
December	Rare	No trace
January 21st	On fresh crop	,, ,,
February	Very common	,, ,,
March	Epidemic	,, ,,
April	,,	,, ,,
May	,,	,, ,,
June	,,	Just appearing

It has been shown that climatic conditions during winter and spring (1921–22) could not be taken as a factor in accounting for the absence of yellow rust this year. Evidently one is forced to the conclusion that the source of infection for the winter crop lies in the uredospores occurring on self-sown plants about autumn time.

Again it has been stated above that wheat sown late in the season (Nov. 30th, 1920) did not get rusted till March 3rd, some six weeks later than the crop sown Oct. 5–7th. It may be pointed out that by the time the seedlings could appear uredospores were very rare so that the chances of an infection at that time were scanty. Besides, from the 12th of December 1920 onwards, there was an exceptionally heavy frost which lasted more than a week. Early in January 1921 again the uredostage was very rare. Keeping these facts in mind one can safely conclude that most probably the infection of the wheat plot under discussion was due to wind-blown uredospores, as there was plenty of yellow rust available by the end of January. The reason why this plot did not show any rust till the beginning of March will be clear from a study of facts discussed under "incubation periods."

As regards the seedlings transplanted outside the laboratory it is interesting to note that up to the middle of July (when the plants were quite ripe and dry) no trace of yellow rust was found on these plants, in all for a period of over eleven months.

For the reasons given above the writer has come to the conclusion that the unprecedented late appearance of yellow rust in the year 1922 in the locality under report was due to the absence of a local source of infection during the autumn and winter of 1921–22. Further, that the source of infection for the rust which was noticed in June was in all probability wind-blown uredospores coming from a place* distinctly cooler or such where the uredo-stage (perhaps under protection) had escaped injury in the summer of 1921. The fact that weather conditions in the summer of 1921 (exceptionally hot as it was) were most unfavourable for the infection of aftermath and self-sown plants by yellow rust will be discussed later.

While summing up it may be remarked that negative results from the cultures of exceedingly susceptible varieties, even when exposed in the open, under conditions of weather undoubtedly favourable for the growth of the fungus go a step further to prove that there is no hereditary source of infection.

(*c*) *Overwintering of uredospores.*

As regards the viability of the uredospores of this rust it may be stated that inoculations conducted with material from the open several times during the winter and spring of 1920–21 gave satisfactory results and the spores invariably showed good germination.

6. Influence of Temperature on the Viability of Uredospores.

Marshall Ward† found that the minimum temperature at which the uredospores of *P. dispersa* on Bromes germinate lies between 10–12° C., the optimum being at or near 20° C., and the maximum between 26–27·5° C. Johnson‡ has stated that the uredospores of *P. graminis* and *P. Rubigovera* can germinate between 2–31° C., the optimum lying between 12 and 17° C.

As far as *P. graminis* is concerned the writer has found that it shows better germination at 29–30° C. than at 2–3° C. At 22–23° C. germination of more than 80 % spores of this rust has frequently been observed. The uredospores of *P. triticina* on the other hand show better germination at 2–3° C. than at 29–30° C.; at 22–23° C. it does not show more than 50 % germination. Lastly *P. glumarum* shows 20–30 % germination

* Early in the year 1922 yellow rust of wheat (though rare) was available in some parts of England. The writer obtained some material from Harpenden in January.

† Ward, H. M., Ann. Bot. xv, p. 560 (1901).

‡ Johnson, E. C., Phytopathology, II, Reviews (1912).

at 2–3° C., not more than 5 % at 22–23° C. and no germination at all at 29–30° C.

On the strength of some further trials the writer has come to the conclusion that the uredospores of *P. glumarum* germinate better than either of the other two rusts at 2–3° C. At 22–23° C. (near the upper limit) those of *P. graminis* germinate better than *P. triticina* but those of *P. glumarum* show very poor germination.

Above 5° C. and up to 20° C. all the three rusts germinate well.

It has been pointed out above that black rust cannot withstand cold, whereas both yellow and brown rusts resist it well. This fact is further borne out by the results of a large number of experiments conducted at different times of the year with uredospores of almost the same age after exposing them to low temperatures in cold storage. The material used in these experiments was cultivated in the laboratory. In all the experiments the controls were kept at laboratory temperatures at which the germination tests were made. The following is a brief summary of the results:

A. Black rust (*P. graminis*).

After exposure to − 6·5 to − 10° C. for 24 hours the uredospores of this rust do not show even 10 % germination and after four to seven days there is a total loss (only one or two spores germinating) of viability.

When exposed to 2·5° C. the spores retain nearly 10 % viability even after one month. They show as much as 50 % germination after exposure to 5° C. for a month.

B. Brown rust (*P. triticina*).

The uredospores of this rust on the other hand show as much as 50–60 % germination after exposure to − 6·5 to − 10° C. for twenty-four hours and even after a week retain nearly 30 % of their viability. After one month's exposure their viability falls to 15 %.

At 2·5° C. and 5° C. the uredospores retain as much as 25 % of their viability after a month's exposure.

C. Yellow rust (*P. glumarum*).

After exposure to − 6·5 to − 10° C. for twenty-four hours the uredospores of this rust germinate quite as well as those of brown rust. After four days they show nearly 25 % germination, but by the end of a week only 10 % spores are viable. At 2·5° C. and 5° C. they retain 15–20 % of their viability after an exposure for one month.

It is essential to point out that the above results are not absolutely constant and that casual variations in the per-

centage of germination may be observed on account of the age of the spores and possibly also on account of the different weather conditions to which the material might be exposed before its removal from the host.

From a comparison of the above results with those obtained from the controls kept at laboratory temperatures the writer has come to the conclusion that in the case of yellow rust there is a rapid fall in the germination capacity of the uredospores. When kept in the laboratory their viability fell down from 80 % to 25 % after a week and by the end of a month the spores showed only 5 % germination, whereas the same rust as already stated keeps nearly 20 % of its viability at 2·5° C. or 5° C. for a month.

The uredospores of black rust, however, show the least amount of impairing in their viability at laboratory temperatures because even after a month they showed as much as 50 % germination.

Brown rust seems to suffer more than the black but much less than the yellow one retaining nearly 30 % of its viability for a period of one month at laboratory temperatures. To elucidate this fact still further two more tests were made in April 1922 for which a constant record of temperature was kept with a thermograph.

Uredospores of all the three rusts were taken fresh from plants inoculated on the same day and kept in the same greenhouse throughout. The temperature of the greenhouse was kept well within favourable limits (4·5–21° C.) and only once or twice rose for a few minutes to 26° C.

1. Spores about ten days old gave the following results when tested at 10·5–15·5° C. (Average for twenty-four hours 12·8° C.)

P. glumarum: nearly 50 % germination
P. triticina: about 60 % ,,
P. graminis: nearly 75 % ,,

2. Spores nearly five weeks old gave the following results at the same temperatures as above and germinated at the same time.

P. glumarum: below 10 % germination
P. triticina: about 30 % ,,
P. graminis: nearly 70 % ,,

These experiments show clearly that uredospores of black rust keep better at ordinary temperatures than either of the other two rusts and that those of yellow rust suffer the greatest loss of viability. By comparing these results with those already quoted one can easily see that it is not only temperature that is responsible for the loss of viability so obvious in the case

of yellow rust, but that the uredospores are on the whole very short lived. It has been shown that even at 5° C. (by no means a high temperature) after one month's time they retain only 20 % of their viability. Besides one finds that a considerable number of spores, even when removed from pustules hardly a week old, look quite dark under the microscope and show a complete absence of the characteristic orange coloured contents. Such spores do not germinate at all. Undoubtedly the uredospores of this rust keep better at lower temperatures.

The uredospores of brown rust are longer lived than those of the yellow one and also keep better than them at warmer temperatures.

It may be pointed out in this connection that considerable difficulty was encountered in keeping the culture of yellow rust in the summer of 1921, as it was abnormally warm. On account of the frequent failure of infection the pure culture that had been kept going since Nov. 1920 was lost. Moreover all attempts to establish a new one were unsuccessful. There was occasionally a little infection after a prolonged incubation but it seldom gave enough material for re-infection. The impaired condition of the uredospores and their subsequent failure to infect the host even when protected from direct sunlight suggests that conditions for the fresh infection of wheat and other cereals were very unfavourable in the summer of 1921. On account of the exceptionally high temperature (the average maximum in the shade during July was as much as 26·2° C.) it is probable that the uredospores suffered great injury. It is no wonder therefore that the rust was practically absent during the autumn and winter of 1921–22, whereas brown rust was noted on wheat and rye throughout that period. The occurrence of *P. simplex* on barley and of black rust on Couch in the autumn of 1921 has already been recorded.

It is interesting to note that with additional precautions it was possible to keep the cultures of both brown and black rusts going even during the summer of 1921.

7. Incubation Periods.

As remarked above Eriksson* has allowed a very variable time limit for the "mycoplasm" to assume a visible form. While writing about one definite season he states, "the period lacking mycelium in the wheat plant's life must be short, e.g. two to three weeks in October after sprouting," because he found fresh pustules of yellow rust in November. In another season he says that this period extended over nearly nine months.

* Eriksson, J., Ann. Bot. xix, p. 55 (1905).

He also states as the chief argument in favour of his hypothesis, that normally the fresh outbreak of rust takes four to six weeks after sowing, and denies, on the strength of this observation, that the source of infection could ever be the uredospores present at the time of sprouting.

This interpretation of Eriksson is based on the opinion that the rust does not take more than eight to ten days from the time of infection to produce pustules.

All the above phenomena, curious as they seem, are easily explicable in the light of the fact that the incubation period is not only not fixed at eight to ten days, but is most variable, and that temperature more than anything else is the regulating factor.

While dealing with incidence it was stated that in October and a part of November 1920 there were a large number of self-sown plants of wheat practically covered with yellow rust growing just outside the cages, where winter wheat was sown in the first week of October. In the beginning of January 1921 the rust was exceedingly rare and was found only as old pustules on a few withered leaves.

On the 20th of January 1921 (about fourteen weeks after sowing) yellow rust was observed on the wheat crop inside the cages. On account of the exceedingly short distance (less than ten yards from some beds) between the rusted plants and the new crop, there could be very little doubt about the source of infection for the young seedlings.

On the 3rd of February last year, some inoculations were tried on wheat seedlings, with material from the rusted plants in the cages, primarily to see if infection does take place in the open during the cold weather and if so how long the pustules take to appear. As a control inoculations were tried in the laboratory and the pot removed to a greenhouse at 60–70° F. The inoculated spots were clearly marked.

1. Pot No. 1. Seedlings inoculated in the open and kept in the open throughout. Pustules on the 36th day.

2. Pot No. 2. Control. Pustules on the 13th day.

Minimum temp. on the 3rd and 4th 23–25° F.
Maximum „ „ „ 43·8° F. } in screen.

Unfortunately the germination tests were not very reliable as most of the spores sank to the bottom of the watch glasses.

The experiment was therefore repeated a week later and the results were as follows:

Pot No. 1. In a heated greenhouse throughout.
Pustules on the 13th day.

Pot No. 2. In a cool greenhouse throughout (45–60° F.).
Pustules on the 28th day.

Pot No. 3. In the open throughout—spores 60–70 % germination.
Pustules on the 34th day.

On the strength of these results one can easily explain why in a colder part of the year the rust took nearly ten to eleven weeks from the probable time of its having gained entry into the young seedlings, to develop pustules. The lowest temperature in the shade for February 1921 was 23° F., the highest was 61° F., the average minimum was 33·3° F. and average maximum 47·6° F.

Hecke* in Austria has shown clearly that the upper limit of the incubation period in the case of yellow rust may go up to five months in a winter which was much more severe than the one we had, except for one week in the middle of December in the year 1920–21. He inoculated wheat on October 28th, which, exposed to winter cold, did not develop pustules till March 28th.

Undoubtedly a very severe winter with more frequent frosts may prolong this period by a few weeks, and if the earlier part of spring also happens to be cold, pustules may not appear till summer actually begins.

It would be useless to enter into the details of all the experiments conducted on the length of the incubation period of this rust under different conditions so only the results of a few conducted in the open will therefore be given.

1.	Wheat inoculated	Feb. 23rd, 1921	Pustules on the	25th	day.
2.	,, ,,	March 24th, 1921	,, ,,	14th	,,
3.	,, ,,	Oct. 26th, 1921	,, ,,	19th	,,
4.	,, ,,	March 3rd, 1922	,, ,,	16th	,,

It is probable that during winter the rust mycelium inside the host grows by fits and starts rather than remains altogether latent, because even in the middle of winter the weather may occasionally be favourable for its growth and fresh pustules may be formed.

That the recurrence of brown rust has a similar explanation will be clear from the following summary of experiments conducted during the spring, autumn and winter of 1921.

1. March 28th:
   - (*a*) Wheat inoculated in the open and kept in the open — Pustules on the 15th day.
   - (*b*) Wheat inoculated in tropical pit at the Botanic Garden — Pustules on the 8th day.

* Hecke, L., Naturw. Zeitschr. f. Forst- u. Landwirtsch. IX, p. 44 (1911).

2. October 26th:
   Wheat inoculated in the open and kept in the open — Pustules on the 20th day.
3. December 9th:
   (*a*) Wheat inoculated in the open and kept in the open — Pustules on the 36th day.
   (*b*) Wheat inoculated in the laboratory — Pustules on the 10th day.

The question naturally arises, as to whether the black rust also can overwinter through its mycelium inside the host that may be infected in autumn. Observations on self-sown plants growing on the same plot unploughed, which had very badly diseased wheat till Oct.–Nov. 1920 show that it is very unlikely, whereas the same plot was full of self-sown plants bearing yellow and brown rusts in plenty. The same might be said about this rust on Couch grass.

The results of the following experiments on the incubation of black rust in the open suggest a similar conclusion.

October 26th, 1921:

Seedlings of rye inoculated in the laboratory at the Botanic Garden and removed to the open after two days—showed no pustules (till Dec. 19th); the inoculated spots became dried and shrivelled.

Control kept in intermediate pit. Pustules 12th day.

It may be remarked, for the sake of comparison, that wheat inoculated with yellow and brown rust on the same date and kept side by side with the above showed pustules within twenty days.

December 19th, 1921:

Rye inoculated in the laboratory at the Botanic Garden and removed to the open after three days; no pustules even after two months when most of the inoculated spots were dead.

Control kept in intermediate pit showed excellent infection within three weeks.

January 31st, 1922:

(*a*) Rye inoculated in intermediate pit and removed to the filmy fern house (very cool) after four days; pustules 24th day. Temp. of pit 40–50° F.; casually up to 60° F. or above in bright sun.

(*b*) Control. Pustules 10th day.

(*c*) Pot removed to the open. Pustules on the 30th day; only three out of twenty spots inoculated, developed pustules.

February 4th, 1922:

(*a*) Wheat inoculated and kept in unheated greenhouse. Pustules 25th day.

(*b*) Wheat inoculated in the laboratory removed to the open after four days. Pustules 27th day.

(*c*) Wheat inoculated in the laboratory and removed to unheated greenhouse. Pustules 22nd day.

(*d*) Control in the laboratory throughout. Pustules 10th day.

Average minimum temperature in shade during February 1922 was 33·5° F.

Average maximum temperature in shade during February 1922 was 48° F.

From the last two experiments it is clear that the mycelium of black rust can survive milder weather and but for the absence of the source of infection might appear on the cereal crops in the earlier part of spring, if weather conditions were favourable. But the source of infection (uredospores) cannot survive the more severe part of winter, and it is highly probable that the mycelium also cannot do so.

As there was no black rust available in the open, the material used for the above experiments was such as had been cultivated in the laboratory. In every case after inoculation the pots were kept indoors for two to four days before removal to the open, to give the fungus a good start. Still it was only in the experiments conducted in more favourable weather that any positive results were obtained.

That the incubation period may be prolonged even under higher temperatures is clear from what happened in one of the experiments last summer (1921).

Out of the six seedlings of Burbanks wheat inoculated with yellow rust in the laboratory on July 26th, and kept indoors under protection from direct sun, only one developed pustules, and that on the seventeenth day; other inoculated spots were dead and shrivelled by then.

Again Red Sudan wheat inoculated on August 8th with yellow rust and removed to the open after two days showed pustules on the eighteenth day but the infection was very poor.

Av. minimum temp. in July 53·8° F. Max. 79·4° F.
,, ,, Aug. 52·7° F. Max. 70·9° F. } in screen.

It may be remarked, that wheat inoculated on the 6th of June with yellow rust developed pustules on the ninth day.

The writer has great pleasure in being allowed to quote from the unpublished work of Dr Burns some interesting data which have a direct bearing on this question.

Dr Burns inoculated very susceptible varieties of wheat with black rust on the 16th of May 1909 at Poona in India, under the shade of a mango tree to protect seedlings from the hot sun and the plants developed pustules on the thirty-fifth day.

Average minimum temp. for May 73·5° F., max. 99·3° F.
„ „ „ June 72·0° F., max. 86·2° F.

Similarly Ward*, Mains† and Fromme‡ have emphasised the influence of higher temperatures on the length of the incubation periods of other rusts. It is only fair to conclude, therefore, that the duration of the incubation period is exceedingly variable and that Eriksson evidently left out of consideration altogether the effect of temperature on the growth of the rust mycelium inside the host.

8. Culture of Rusts at comparatively low temperatures.

During the spring of the year 1922 some further experiments were conducted with the object of finding out the comparative duration of the incubation period in the case of all the three rusts cultivated at temperatures regulated to lie above the freezing point but not allowed to go very high. A constant record of the temperature of the greenhouse was kept with a thermograph and the average of daily temperatures was worked out by noting six readings per day. In all these experiments seedlings of Red Sudan wheat (which is very susceptible to all the three rusts) were used. The germination of spores was in every case found to be very good.

The lowest temperature recorded during the first experiment was 32·5° F. and the highest 72° F. In the second experiment it was 37° F. and 78° F. respectively. In the third it was 43° F. and 77° F. The weather during the course of the fourth experiment however was very warm and although the experiment was conducted in a cool greenhouse the lowest temperature recorded was 50° F., the highest being 82° F.

The results of these experiments are shown in the following table.

No.	Date at which the experiment was started	Range of average daily temperatures for the period. (The average for the whole period is shown within brackets)	Number of days after which pustules appeared		
			Yellow rust	Brown rust	Black rust
		° F.	Days	Days	Days
1	March 2nd	38·6 to 50·6 (40·0)	12–13	17–18	21–22
2	April 3rd	43·5 to 58·0 (50·8)	11–12	13–14	14–15
3	„ 14th	48·5 to 58·8 (54·1)	8–9	8–9	11–12
4	May 18th	59·1 to 71·8 (66·5)	12–14	8–9	8–9

* Ward, H. M., Ann. Bot. xv, p. 560 (1901).
† Mains, E. B., Amer. Journ. Bot. iv, p. 179 (1917).
‡ Fromme, F. D., Bull. Torrey Bot. Club, xl, p. 501 (1913).

The above results clearly show that the optimum temperatures for the growth of the mycelium in the three rusts are different. Black rust undoubtedly flourishes better at higher temperatures and cold has a pronounced inhibitory effect upon its growth. The brown rust occupies an intermediate position, whereas the yellow one can flourish at low temperatures.

Further the results of the first experiment suggest a possible explanation of the sequence in the appearance of the yellow and the brown rusts. It has been pointed out that in this country yellow rust usually appears before the brown one. The reason why black rust does not appear till late in the season is that the source of fresh infection of cereals lies in the aecidiospores on barberry and the aecidial stage does not appear till late in spring.

9. Infection from Collateral Hosts.

Eriksson* has pointed out that the spread of rust from one kind of cereal or grass to another is considerably restricted on account of specialization of parasitism.

Similarly Butler† has remarked that wild grasses play no part in carrying rust in the main wheat growing tracts of India.

Stakman and Piemeisel‡ have stated that grasses aid materially in the spread of black rust on cereals. These authors have also remarked that, even if overwintering of this rust on grasses is infrequent, grasses may still be important because they may be nearer barberry bushes than are the grain fields.

As far as this locality is concerned the overwintering of black rust, whether on cereals or grasses, is very doubtful. The possibility of the fresh outbreak of the rust each year due to infection from other cereals or grasses is therefore out of the question. Besides it has been stated above that in wild grasses it has been observed only on Couch grass.

Yellow rust has been observed by the writer only once on *Agropyron repens* and *Agropyron caninum* but Miss Sampson sent some specimens of *Dactylis glomerata* from Aberystwyth which were found to be infected.

Speaking generally one might state that on account of a more rigid specialization of parasitism in the case of both brown and yellow rusts the possibility of other hosts playing a part in their perpetuation is limited. A detailed discussion of this question as far as this locality is concerned will be taken up in the second part of this paper.

* Eriksson, J., Bot. Gaz. xxv, p. 26 (1898).
† Butler, E. J., Fungi and Disease in Plants (1918).
‡ Stakman, E. C. and Piemeisel, F. J., Journ. Agr. Res. x, p. 429 (1917).

PART II.

SPECIALIZATION OF PARASITISM.

In recent years considerable work has been done on the biologic forms of rusts on cereals. In Europe Eriksson*, Eriksson and Henning† and Jaczewski‡ have made extensive investigations. In the United States Carleton§, Freeman and Johnson‖, Pritchard¶ and quite recently Stakman and Piemeisel** have worked on different aspects of the question.

On account of the absence of any definite information on the subject for this country, and also on account of the bearing it has on the problem of the annual origin of rusts on wheat it was desirable that some investigations should be made. Further it is clear that in spite of the general agreement in the results obtained by various investigators there are interesting differences which have indicated the necessity for such work in different regions.

The present note deals with an account of some of the experiments conducted with the uredospores of black, brown and yellow rusts obtained on wheat in this locality. It also gives an account of some work done with the black rusts of barley and Couch grass (*Agropyron repens*) and the brown and yellow rusts of barley.

In order to avoid the use of impure material in the experiments a pure culture was invariably first established. Inoculations with different biologic forms were made in separate greenhouses.

Black rust from wheat (P. graminis tritici).

This biologic form was observed on tillers and self-sown plants of wheat on the Little Joss plot in October 1920 about a couple of months after harvest. It was established on wheat in November and since then its culture has been kept in the laboratory. The following is the table of results of inoculations with that material after its culture on wheat for six generations. In experiments (2) and (3) material used was respectively twelve

* Eriksson, J., Ark. f. Bot. I, p. 139 (1903); Ber. Deutsch. Bot. Gesell. XII, p. 292 (1894); Centralbl. f. Bakt. Bd. IX, Abt. 2, p. 590 (1902); Zeitschr. f. Pflanzenkrankh. IV, p. 66 (1894).

† Eriksson, J. and Henning, E., Die Getreideroste (1896).

‡ Jaczewski, A. A., Zeitschr. f. Pflanzenkrankh. XX, p. 321 (1910).

§ Carleton, M. A., U.S. Dept. Agric. Div. Veg. Phys. and Path. Bull. No. 16 (1899)

‖ Freeman, E. M. and Johnson, E. C., U.S. Dept. Agr. Bur. Plant. Indus. Bull. p. 216 (1911).

¶ Pritchard, F. J., Bot. Gaz. LII, p. 169 (1911).

** Stakman, E. C. and Piemeisel, F. J., Journ. Agr. Res. X, p. 429 (1917).

and thirteen generations old. The spores showed good germination in every case.

Date	Hosts inoculated				
	Wheat	Barley	Rye	Oats	*Agropyron repens*
1921 Aug. 8th	*R* $\frac{4}{6}$ Heavy *En* $\frac{1}{6}$ Very weak	B_{39} $\frac{3}{6}$ Moderate B_{25} $\frac{2}{6}$ Weak	$\frac{0}{6}$	E_{82} $\frac{0}{6}$	$\frac{0}{10}$
1922 Feb. 10th	*R* $\frac{8}{8}$ Heavy *J* $\frac{4}{7}$ Moderate	B_{39} $\frac{3}{6}$ Moderate B_{7} $\frac{9}{18}$ Weak B_{25} $\frac{2}{6}$ Weak	$\frac{0}{8}$		
March 7th	*R* $\frac{8}{8}$ Heavy *En* $\frac{4}{13}$ Very weak	B_{7} $\frac{8}{11}$ Moderate	$\frac{0}{10}$	E_{82} $\frac{0}{10}$	

NOTE. In the above table *R*, *J* and *En* stand for varieties of wheat called Red Sudan, Little Joss and Einkorn respectively. B_{39} stands for a variety of Mesopotamian barley, B_7 for Archer barley, B_{25} for Webb's winter 6-row barley. E_{82} stands for a strain of Thousand dollar oats. The denominator in the fractions indicates the number of seedlings inoculated and the nominator the number actually infected. The nature of infection is also indicated. In the case of Einkorn wheat the pustules were exceedingly small and appeared much later than those on other hosts.

Seedlings of *Lolium perenne, Arrhenatherum avenaceum, Dactylis glomerata* and *Hordeum jubatum* were also inoculated along with others in the first experiment but none of them was infected.

Eriksson* has stated that in Sweden, barley, rye and oats are sometimes weakly infected by the black rust of wheat. The results of Stakman and Piemeisel† are quite in agreement with Eriksson's except that barley has been found to be a congenial host by these writers. As regards the wild grasses referred to above, Stakman and Piemeisel have stated that *Hordeum jubatum* is one of the hosts infected in nature by this biologic form and that *Agropyron repens* can be weakly infected by it. The same authors have observed that even rye is found infected by it in nature.

* Eriksson, J., Ber. Deutsch. Bot. Gesell. XII, p. 292 (1894); Centralbl. f. Bakt. Bd. IX, Abt. 2, p. 590 (1902); Zeitschr. f. Pflanzenkrankh. IV, p. 66 (1894).

† Stakman, E. C. and Piemeisel, F. J.. Journ. Agr. Res. X, p. 429 (1917).

Carleton* has however stated that rye and species of *Agropyron* are not infected by this form. Again Jaczewski† says that barley and *Lolium perenne* can be infected, but rye, oats and *Dactylis glomerata* are immune. Lastly Butler and Hayman‡ have also found that in India this form can infect barley.

This form has not been found in the locality under report on any other host except wheat. It will be clear from the above results that the specialization of this form is quite rigid and that among the cereals it infects only wheat (its original host) and barley. The capability to infect wild grasses is doubtful on account of the small number of trials made.

Amongst the varieties of wheat tried Red Sudan is exceedingly susceptible to this rust. Other varieties, Burbanks, Wilhelmina, Little Joss, Yeomen, Dreadnought, are all moderately susceptible. Squareheads master and American club are only weakly infected but Einkorn wheat is very highly resistant, though not altogether immune.

Amongst the varieties of barley tested, B_{39} (the Mesopotamian variety exceedingly susceptible to yellow rust) has been found to be far more susceptible than B_7, B_{25} or B_{66}. In order to find out if barley was a "bridging host" for this form seedlings of rye, oats, barley and Red Sudan wheat were inoculated with the material cultivated on barley (B_{39}). It is interesting to note that wheat (the original host) and barley were infected but rye and oats were not.

In February and March 1922 the writer inoculated on two different occasions seedlings of wheat, barley, rye and oats with uredospores of this form after their cultivation on Red Sudan wheat (exceedingly susceptible) for thirteen and fourteen generations respectively. Wheat and barley became infected but rye and oats were quite immune.

The writer is in complete agreement with Stakman, Piemeisel and Levine§ and Stakman, Parker and Piemeisel‖ who have stated that the pathogenicity of biologic forms is not changed by bridging hosts or by association with a given host. The form under discussion did not acquire any new parasitic capability even after its continuous cultivation for over sixteen months on Red Sudan wheat. Puttick¶ has also pointed out that the parasitic

* Carleton, M. A., U.S. Dept. Agric, Div. Veg. Phys. and Path. Bull. No. 16 (1899).

† Jaczewski, A. A., Zeitschr. f. Pflanzenkrankh. xx, p. 321 (1910).

‡ Butler, E. J. and Hayman, J. M., Mem. Dept. Agric. India, Bot. Ser. i, No. 2 (1906).

§ Stakman, E. C., Piemeisel, F. J. and Levine, M. N., Journ. Agr. Res. xv, p. 221 (1918).

‖ Stakman, E. C., Parker, J. H. and Piemeisel, F. J., Journ. Agr. Res. xiv, p. 111 (1918).

¶ Puttick, G. F., Phytopathology, xi, p. 205 (1921).

capabilities of the biologic forms that he worked with were constant.

Black rust of Agropyron repens (P. graminis secalis).

This biologic form has been found only on Couch grass (*Agropyron repens*) in this locality. It is probable, however, that the form found on barley which will be dealt with later is identical with it. The following table gives the results of inoculations with uredospores. In experiment No. 1 the material used was such as had been cultivated on Couch grass for one generation. In other experiments the material used had been cultivated on rye for two, three, five and six generations respectively.

Date	Hosts inoculated				
	Agropyron repens	Rye	Wheat	Barley	Oats
1921					
Aug. 11th	$\frac{5}{10}$ Moderate	$\frac{6}{6}$ Heavy	*R* $\frac{4}{7}$ Very weak *En* $\frac{0}{6}$	*B*39 $\frac{6}{6}$ Good *B*25 $\frac{2}{6}$	$\frac{0}{6}$
Aug. 30th	—	$\frac{4}{6}$ Heavy	*R* $\frac{2}{4}$ Very weak	*B*39 $\frac{3}{3}$ Good	$\frac{0}{4}$
Sept. 13th	—	$\frac{5}{6}$ Heavy	*R* $\frac{2}{6}$ Very weak	*B*37 $\frac{3}{7}$ Weak	$\frac{0}{4}$
1922					
Jan. 24th	—	$\frac{27}{29}$ Heavy	*R* $\frac{7}{11}$ Very weak *J* $\frac{0}{10}$	*B*7 $\frac{8}{14}$ Weak	$\frac{0}{12}$
Feb. 25th	—	$\frac{12}{16}$ Heavy	*R* $\frac{8}{15}$ Very weak *J* $\frac{0}{10}$	*B*7 $\frac{5}{11}$ Weak *B*25 $\frac{4}{10}$ Weak	—

Note. The varieties used are the same as tried with the biologic form of wheat. For the cultivation of this form seedlings of rye were found to be more convenient than those of Couch grass (the original host).

Along with the first experiment seedlings of *Dactylis glomerata*, *Lolium perenne*, *Arrhenatherum avenaceum* and *Hordeum jubatum* were also inoculated but none of them became infected.

Eriksson* has found that this form infects rye, *Agropyron repens*, *Hordeum jubatum*, barley and some of the grasses but not wheat and oats. Eriksson and Henning† have recorded that inoculations with material from rye infected rye and barley but not oats and wheat. Jaczewski‡ in Russia has remarked that *Dactylis glomerata* can be infected and that barley, wheat and oats are immune.

* Eriksson, J., Ber. Deutsch. Bot. Gesell. XII, p. 292 (1894).
† Eriksson, J. and Henning, E., Die Getreideroste (1896).
‡ Jaczewski, A. A., Zeitschr. f. Pflanzenkrankh. XX, p. 321 (1910).

Again Stakman and Piemeisel* in the United States have stated that this form is found on *Agropyron repens*, *Hordeum jubatum*, barley, rye and some other hosts in the field. The same authors succeeded in infecting barley easily but not *Dactylis glomerata*, *Lolium perenne* and *Arrhenatherum avenaceum*, etc. As regards wheat these authors have remarked that it is very rarely or scarcely at all infected by this form. Out of four hundred and fifty-four trials according to their data only three gave positive results. These authors state that oats also can be rarely and weakly infected.

It will be seen that it has been possible to infect *Agropyron repens* (the original host), rye, barley and contrary to what has been reported from other regions, even wheat (at least the variety Red Sudan). Oats are not infected. About the grasses mentioned above the writer is doubtful (on account of the small number of trials) if they can be infected by this form.

As regards Red Sudan (wheat) infected by this form positive results were obtained in twenty-nine cases out of fifty with undoubtedly a pure culture (after cultivation on rye for twelve generations). The size of the uredo-sori developed on wheat was invariably much smaller than those on rye or barley.

Inoculations with material as many as ten and eleven generations old was also tried on other varieties of wheat—Little Joss, Burbanks, Yeomen, Dreadnoughts, Squareheads master, American Club and Red Sudan and rye (as controls). Only rye and Red Sudan became infected and all other varieties of wheat were found to be immune. This fact can easily be explained on account of the exceedingly susceptible nature of Red Sudan. From over 50 % cases of successful infection one cannot but conclude that the specialization of this form is not absolutely rigid as far as wheat is concerned. It is not possible at this stage to make any definite statement on the possibility of wheat taking infection from Couch grass or rye in the open. As far as barley is concerned there is not the slightest doubt that Couch grass, on account of its plentiful distribution, may not only be responsible for the spread, but on account of its proximity to barberry may even be the cause of the fresh outbreak of black rust on that cereal.

As shown in Table, p. 170, the variety most susceptible to this form is B_{39} (Mesopotamian barley). The other varieties are not so susceptible. It is interesting to note that the same varieties are only moderately susceptible to the black rust of wheat also.

This form too was cultivated on barley (B_{39}) for two generations and inoculations were made on rye, barley, Red Sudan and oats with that material. Rye, barley and Red Sudan were

* Stakman, E. C. and Piemeisel, F. J., Journ. Agr. Res. x, p. 429 (1917).

infected but oats were immune showing that barley cannot change the infection capability of this form either.

Black rust of barley.

In August 1921 this rust was found at the farm with the infected bushes of barberry referred to in Part I. Black rust was noticed on *Agropyron repens* a few weeks earlier on the same farm.

The following table shows the results of inoculations with a pure culture of this form.

Date	Original host	Immediate host	Hosts inoculated				
			Barley	Rye	Wheat	Oats	*Agropyron repens*
1921 Sept. 4th	Barley Archer	Barley B_{39}	B_{39} $\frac{3}{4}$ Moderate	$\frac{4}{4}$ Heavy	$\frac{0}{5}$	$\frac{0}{4}$	$\frac{3}{4}$ Moderate

Eriksson* has stated that the black rust of barley can infect rye and barley but not oats and wheat. Jaczewski† on the other hand has found that uredospores from barley can infect barley, Couch grass and wheat but not oats and rye. According to his results the forms on barley and wheat seem to be identical in Russia.

Again Stakman and Piemeisel‡ have remarked that black rust of barley infects wheat more readily than rye and that oats are not infected by it.

It has been shown above that the black rust of *Agropyron repens* can infect wheat (Red Sudan) and rye, but the black rust of wheat cannot infect rye. Since the black rust of barley can infect rye readily and also Couch grass, it seems highly probable that this form is different from the form on wheat. Most probably the form on barley is identical with the form on *Agropyron repens* (f. sp. *secalis*). In the last table it has been shown that this form failed to infect wheat (Red Sudan) which is infected by the form on *Agropyron repens*. Unless the form on barley too can infect Red Sudan wheat its exact relation with f. sp. *secalis* cannot be established. Unfortunately the culture of this form was lost and on account of the uredo-stage being so rare later on it could not be re-established. Further trials with this were not possible.

It is obvious that apart from slight local differences speciali-

* Eriksson, J., Ber. Deutsch. Bot. Gesell. XII, p. 292 (1894); Zeitschf. f. Pflanzenkrankh. IV, p. 66 (1894).

† Jaczewski, A. A., Zeitschr. f. Pflanzenkrankh, XX, p. 321 (1910).

‡ Stakman, E. C. and Piemeisel, F. J., Journ. Agr. Res. X, p. 429 (1917).

zation is not quite so fixed as far as black rust is concerned. Barley, as has already been pointed out by Stakman and Piemeisel, is infected by all the six forms that these authors have worked with. Again rye has been reported in other regions to be infected by the form on wheat. In this locality wheat has been repeatedly infected with the form on Couch grass. It seems likely that in every class of cereals there are certain varieties, which are undoubtedly susceptible to one or more biologic forms of this rust, other than the one to which they are said to be restricted. Probably the differences in specialization recorded from different regions are largely due to the fact that the various investigators have been working with different varieties. To illustrate this fact one may say that some more susceptible variety of rye may be infected by the form on wheat in this locality. Again the variety of wheat (Red Sudan) may be infected by the form on rye or Couch grass in other countries too.

Brown rust of wheat (P. triticina *Erikss. and Henn.*).

Eriksson* has found that this form can sometimes infect rye. The writer made some inoculations with a pure culture on wheat, rye, barley and *Agropyron repens*. Barley and *Agropyron repens* were not infected, wheat showed very good infection and out of twenty-one seedlings of rye three were infected. The results are thus in complete agreement with those of Eriksson as regards the casual infection of rye with the brown rust of wheat.

Brown rust of rye (P. dispersa *f. sp.* secalis *Erikss. and Henn.*).

This form has been found by Eriksson* to be altogether specialized on rye. The same has been found to be true in this locality. Inoculations with a pure culture were made in August 1921, and in both the trials it was found that barley, wheat (Red Sudan) and *Agropyron repens* were not infected, whereas rye inoculated on both occasions showed heavy infection.

The dwarf brown rust of barley (P. simplex *Erikss. and Henn.*).

Some inoculations were made in August 1921 with a pure culture of this rust, on wheat (Red Sudan), rye, *Agropyron repens* and barley; only barley was infected. This rust too seems therefore to be quite specialized.

Yellow Rust.

In this rust also there is strict specialization of the different biologic forms as pointed out by Eriksson†. The writer

* Eriksson, J., Ann. Sc. Nat. sér. 8, IX, p. 241 (1899).
† Eriksson, J., Ber. Deutsch. Bot. Gesell. XII, p. 292 (1894).

made several inoculations with a pure culture of the yellow rust of wheat and found that barley and rye are not infected by it. Some experiments with the yellow rust of barley were conducted in the summer of 1921, but on account of very hot weather the infections were unsuccessful even on barley itself, and the pure culture was lost. Yellow rust was noticed on rye in this locality last summer (1921) but an attempt to establish a culture failed on account of the great heat, nor was it possible for any work to be done with the form on Couch grass.

I wish to express my warmest thanks to Mr F. T. Brooks, under whose supervision these investigations have been made, for advice and the interest he has taken in their progress. I am also very grateful to Mr F. L. Engledow and Mr S. F. Armstrong of the School of Agriculture for having provided me with seeds of the different varieties of cereals used in the experiments.

August 1922.

10. Summary.

Part I.

(*a*) *Black rust* (P. graminis).

(1) Fresh uredo-sori of this rust are not found after the commencement of winter. Uredospores in old pustules exposed to winter cold soon lose their viability.

(2) Observations and experiments show that this rust cannot overwinter in this locality even as mycelium inside the host plants.

(3) Direct infection of wheat by sporidia is not possible.

(4) There is no evidence to show that this rust can originate from "mycoplasm" or from intra-seminal sori.

(5) The annual outbreak of this rust can be explained only by fresh infection through aecidiospores produced on barberry.

(*b*) *Brown and yellow rusts* (P. triticina *and* P. glumarum).

(6) Fresh uredo-sori of the brown rust of wheat and the dwarf rust of barley are found during the greater part of winter. Uredospores from the open during winter always show good germination.

(7) Fresh uredo-sori of the yellow rust of wheat were found during the greater part of the winter of 1920–21. Uredospores from such pustules always germinated well.

(*c*) *Incubation period.*

(8) For the annual recurrence of rusts the factor of the greatest importance is the occurrence of plenty of uredospores on self-sown plants and tillers at the time when the autumn sown crop appears. The infection of young seedlings is followed by

a comparatively long incubation, the exact duration of which is dependent upon conditions of weather—temperature being the chief regulating influence.

(9) The length of the incubation period is not only not fixed at eight to ten days but is very variable. Although the last two seasons were comparatively mild the incubation period in some of the experiments was prolonged to five to six weeks.

(10) These facts also suggest a satisfactory explanation of the phenomenon of spontaneous outbreaks (so characteristic of yellow rust) over large areas within the space of a few days.

(11) Cultures of all the three rusts at comparatively low temperatures show the shortest incubation period for the yellow rust and the longest for the black one. A clear difference between the duration of the incubation periods of yellow and brown rusts suggests the cause of the usually earlier appearance of the former.

(*d*) *Influence of temperature on the viability of uredospores and the growth of the mycelium.*

(12) The uredospores of black rust retain their viability at comparatively high temperatures. Those of yellow rust suffer most from heat and are more viable when kept at lower temperatures. The uredospores of brown rust however combine the powers to resist cold as well as heat.

(13) The uredospores of yellow rust are short lived and are impaired at temperatures which both black and brown rusts can withstand.

(14) Higher temperatures are inhibitory to the growth of the mycelium of yellow rust, and therefore prolong the duration of the incubation period, and may even kill the mycelium.

(15) Some of the most susceptible varieties of wheat and barley even when exposed in the open for over ten months do not become affected by yellow rust if the viability of the uredospores (the only source of infection) is impaired.

(16) The observations and experiments recorded in this paper deny all possibility of an hereditary source of infection and offer an adequate explanation of the annual recurrence of these rusts.

Part II.

Inoculation experiments with pure cultures of the following biologic forms showed that:

(1) The black rust of wheat can infect only wheat and barley, but does not infect rye and oats.

(2) The black rust of Couch grass readily infects Couch grass, rye and barley, and contrary to what has been observed at other places can also infect one variety of wheat (Red Sudan).

(3) The black rust of barley infected barley, rye and Couch grass but failed to infect wheat. This form is different from that on wheat and probably is identical with that on Couch grass.

(4) As far as black rust is concerned specialization is not quite so fixed as has been recorded elsewhere. In this locality Couch grass infected with this rust may be a source of danger to barley.

(5) The brown rust of rye is rigidly specialized to its host.

(6) The brown rust of wheat casually infects rye.

(7) The dwarf rust of barley and the yellow rust of wheat cannot infect other cereals.

THE LITERATURE ON THE CLASSIFICATION OF THE HYSTERIALES.

By G. R. Bisby.

The group of the Ascomycetes known as the Hysteriales is at present commonly considered to include a large number of forms characterized by fruit-bodies (known usually as perithecia or apothecia) which open tardily by a longitudinal fissure. The accretion of forms into this group is reflected by Saccardo, who gives, in volumes II, IX, XI, XIV, XVI, XVII and XXII of the *Sylloge Fungorum*, seven hundred and three references to the Hysteriaceae and thirty-eight to the Hemihysteriaceae, included in a total of fifty-one genera. Eliminating duplicate, varietal, and revoked references, there remains a total of some six hundred and seventy species in forty-six genera in these two related groups in the *Sylloge*, and many species have of course been described too recently to be included.

The writer has gone over the scattered literature in an attempt to set forth the ideas of classification held by various workers on this group. The knowledge of these fungi has, of course, undergone much development during the past two centuries, but the Hysteriales are still comparatively poorly understood.

Few early botanists devoted much attention to the fungi, and even when fungi were noted, the conspicuous forms naturally received first attention. The hundreds of Hysteriales, Pyrenomycetes, Lichens, and other small, undescribed fungi which occurred in the woods and fields would either be unnoticed, or could be described only vaguely prior to the close of the 18th century. Certain Hysteriales and other fungi were included in

some of the early descriptions of lichens. Thus Ray (1, p. 71) may have observed one of the Hysteriales; he gave the following description in 1724: "48. Lichenoides crusta tenuissima, peregrinis velut litteris inscripta. Found by *Dr Doering* at *Westerham*, a little beyond the Schoolhouse on a Tree in the Lane, that leadeth to *Querry's* in *Kent*." There is nothing very distinctive about this description, which would probably more aptly apply to certain lichens, but the Latin diagnosis was quoted verbatim by Micheli, Dillenius, Linnaeus, Haller, and others, and after modification and elaboration came finally to be involved in the synonymy of *Hysterium pulicare*, and also in the synonymy of the lichen *Graphis scripta*. In other words, various writers following Ray considered that the material they examined agreed with the general description Ray had given, or with the descriptions subsequent writers had elaborated from Ray's description and an examination of other material, until finally a definite plant could be recognised.

Micheli (2, p. 102) described: "7. Lichen crustaceus, tartaricus, arboribus adnascens, niger, & veluti deustus, receptaculis florum transverse oblongis, & quasi reniformibus non intortis, brevibus. Tab. 54. fig. 2. Per ambulacrum Imperialis villae in Ilicum cavernulis late se diffundens." This is referred to by Lightfoot and others as noted below. Micheli quoted Ray in No. 9, and noted "receptaculis florum sub cute erumpentibus, nigricantibus, transverse oblongis, varie divarigatis, & compressis." His numbers 10 and 11 are noted by Dillenius and Linnaeus.

Dillenius (3, p. 125) quoted Ray, gave figures, and noted: "Per tenui constat crusta albicante, cui variae figurae lineae inaequales, nigrae,...sunt....Nec tubercula nec scutellae aliquae mihi visae sunt." He stated that this plant occurred on elms and oaks and considered Micheli's Nos. 9, 10 and 11 to be no more than varieties. Dillenius followed with "2. Lichenoides punctatum & rugosum nigrum. The black dotted wrinkled Lichenoides. Crusta est tenuissima candida, cui maculae transversim innascuntur nigrae, pro arborum aetate & magnitudine majores & minores, nunc ex punctis elevatis, velut tuberculis quibusdam parvis, nunc ex lineis oblongis, tanquam rugis, dense congestis conflatae. Quae rugae vel non, vel parum ramosae sunt, linea vel sulco in medio (per lentem) destitutae, secus ac in praecedenti specie. A qua porro differt, quod puncta & rugae densius congestae sint." This form he reported on beech and oaks where the bark was still smooth and equal.

Haller (4, p. 188) listed: "Lichen bivalvis de rimis efflorescens, ater, characteristicus," followed by Ray's words, but with reference only to "Dill. t. 18. f. 1."

Linnaeus (5, p. 343) in 1745 merely brought together these

previous descriptions in his "941. Lichen leprosus albicans, lineolis nigris characteriformibus," and quoted Ray, Dillenius' No. 1, and Micheli's No. 9, and included Micheli's Nos. 10 and 11 (following Dillenius) as varieties. In *Species plantarum* (VI, p. 1140) Linnaeus gave this the binomial *Lichen scriptus*, and the name *Lichen rugosus* to Dillenius' No. 2, with the description "Lichen leprosus albicans lineolis simplicibus punctisque nigris confertis."

References to these species of Linnaeus were given in a number of the Floras that were so commonly published by various workers who followed Linnaeus, but in most of these no new data that enlighten us regarding the Hysteriales are found.

Lightfoot (7, pp. 800, 801) in 1777 gave the following suggestive notes:—"[Lichen] scriptus. 1. Lichen leprosus albicans, lineolis nigris ramosis characteriformibus. Sp. pl. 1906. Micheli gen. t. 56 f. 3. Dillen. musc. t. 18. f. 1.

"On the smooth bark of trees, frequent, as on beech, oak, hornbeam, etc. This is readily distinguished by its black fructifications, resembling small oriental characters, which, under the microscope, appear to be longitudinally wrinkled.

"There are two very remarkable varieties of this lichen, which might perhaps be more properly considered as distinct species:

"Hebraicus α. The first has large black, smooth fructifications, standing in high relief, of no regular figure, but from their size and thickness have a rude likeness to Hebrew characters. *Fig. non inveno.*

"Pulicaris β. The other consists only of small black, oval tubercles each about the size of a flea, having a longitudinal furrow on the back. It grows generally in the crevices of the bark of old oaks. The figure of Micheli agrees with it. Gen. pl. t. 54. ordo 37. f. 2.

"Neither of these two last are in Dillenius's collection at Oxford." The specimens upon which Lightfoot based the above descriptions could not be found in the Lightfoot herbarium at Kew.

Wiggers (8, p. 86) transferred *Lichen scriptus*, *L. rugosus*, and others to his genus Verrucaria. Hoffman (9, p. 14) made Lightfoot's varieties into species. His figures (t. 3. fig. 2, e, f) are uncertain.

Bulliard (10, p. 187) figured and described rather indefinitely *Variolaria corrugata* on the bark of branches, and (p. 170) *Hypoxylon ostraceum*, both of which may have been Hysteriales.

Tode (11) in 1783 described *Acrospermum unguinosum* but the genus is usually stated to date from his 1790 work. In 1784 (12) he applied the name Hysterium to a fungus which he described

as "Fungus labiatus sessilis per labia distenta semen nudum superficiale ejaculans" with the species *H. quadrilabiatum*. He gave this the common name "Venusschwämme." No previously described similar species were mentioned. In 1790 (13, p. 8) *Acrospermum compressum* and two other new species were described and (p. 30) *Hysterium bilabiatum*. In 1791 (14, pp. v, vi, 3–5) he explained that *H. quadrilabiatum* proved to be, on further study, a weathered or altered Sphaeria, and he was uncertain about *H. bilabiatum*, so he re-described the genus as "Fungus oblongo-acuminatus, cavus, sessilis, linea transversali superne findendus, seminibus globoso-ecaudatis, discum obducentibus" and observed "medium hoc genus inter Pezizas & Lichenes." He referred to *Lichen scriptus* L. and to Wiggers and Hoffman. Three new species were described and figured. The genus Hysterium is usually stated to date from this revised description of it in 1791.

Bolton (15, p. 124, fig. cxxiv) described *Sphaeria sulcata*, giving Lightfoot's variety β as a synonym, and observed that "This is a small, black, oblong tubercle, the size of a small flea; it is prominent, and has a deep furrow along the back, from end to end, by which it seems as if cloven in two, but the ends are joined together at the base....The plant under the double magnifier appeared like a bivalve shell, when closed and seen in front...on twigs and branches of ash trees, when in decay." He gave good figures of the external appearance of the fungus.

Acharius (16, p. 20) in 1798 described *Lichen alneus* on the bark of old Betula and Alnus, and *L. clatinum*, both of which subsequent authors considered to be Hysteriales. Under *Lichen scriptus* he listed a total of twenty-nine author citations. These included, in addition to certain authors noted above, those who had compiled Floras and included this name. In 1810 (17, p. 265) Acharius changed the name to *Graphis scripta*. In 1814 (18) he described several species of Opegrapha, which were later transferred to species of Hysteriales.

Persoon (19, p. 83) listed *Hysterium nigrum* Tode, *Variolaria corrugata* Bull. and *Lycoperdon vulvatum* Latourette as synonyms of his *Hysterium quercinum*, and included, without synonyms, three other new species of Hysterium. In *Synopsis methodica fungorum* (20, p. xii, 98, etc.) he included Hysterium with Sphaeria, etc., in his order Sclerocarpi of the class Angiocarpi. Hysterium is described briefly as "perithecium oblongum, rima longitudinali dehiscens," and nineteen species are given. *Lichen scriptus* β *pulicaris* Lightf. and "Mich. Nov. gen. pl. t. 50, f. 2" are given as synonyms for *H. pulicare*, *Sphaeria sulcata* Bolt. for *H. Fraxini*, and the synonyms given above for *H. quercinum*, except that Latourette's species is omitted. (Latourette had

listed the name without description, in *Chloris Lugdunensis*, 1785). The genus Hysterium was considered in a broad sense by Persoon, and subsequent workers, especially upon examining the spores, placed certain of the species in other genera. Certain species of Xyloma as described by Persoon were also subsequently removed to the Hysteriales. Species of Acrospermum were placed by Persoon in the genus Clavaria. In *Mycologia Europaea* (21, p. 331) Persoon described Tryblidium.

Persoon brought together a number of similar fungi into the genus Hysterium, and made a considerable advance in systematizing the knowledge concerning certain forms which had previously been overlooked or placed in the lichens or in various genera of fungi. The workers up to, and even including, Persoon, had naturally not been able to make much headway with these fungi, since the internal, microscopic characters were still almost unknown. Rapid progress was soon made and it is unnecessary to attempt to follow the specific, or even later generic, changes that came to be made, but the systems of classification and development of knowledge may be followed in a general way.

Sowerby (22) illustrated and gave notes on several forms. Albertini and Schweinitz (23) followed Persoon.

De Candolle (24, p. 280) gave the name "Hypoxyla" to a group of genera including Sphaeria, Xyloma, Opegrapha, Verrucaria, Hysterium and Hypoderma. This last genus was described as new, for subepidermal Hysteria, and five species of Hysterium were transferred to it.

Muehlenberg (25, p. 101) listed *Glonium stellare* without description. The genus was later described by him in Fries (27). Nees (26, Ueberblick, pp. 72–86) included Antennaria, Hysterium, Sphaeria, Thelobolus and Nemaspora in his group Myelomyci (Kernschwämme).

Fries (27) in his *Systema Mycologicum* proposed the name Pyrenomycetes, with Xylomyci Willdenow, Sclerocarpi Persoon, Hypoxyla De Candolle, and Goniomyci and Myelomyci Nees, as synonyms. He included as orders Phacidiacei, Sphaeriacei, Cytisporei, and Xylomacei, but noted that the Phacidiacei were aberrant in that they were often cupulate, but had the general habit of Pyrenomycetes. Under the Phacidiacei he included Hysterium Tode (including Hypoderma DC.) with forty-eight good species, three more doubtful, four listed as aberrant, and some twenty other species were transferred, discarded, or noted; Glonium (under Muehlenberg's name) with *G. stellatum*: Actidium with two species; and the genera Rhytisma, Phacidium and Excipula. Lophium he included under Sphaeriacei. Acrospermum was placed in the Sclerotiacei of the Gasteromycetes.

In 1825 (28) Fries altered somewhat his classification of the Pyrenomycetes. He included under Sphaeriacei a sub-order Dichaenei with his genera Dichaena and Ostropa, and under Phacidiacei the sub-orders Patellarei including, among others, Tryblidium; Cenangei; Cliostomei including Glonium, Lophium and Actidium; and Phacidiei, including Hysterium. He noted for Hysterium that "Nominis Auctor est Tode, sed nulla illius species huc pertinet." In reality, as von Hoehnel (76) points out, the name should be cited as Hysterium Fries.

In these two works and in *Observationes Mycologicae* (29) and *Elenchus fungorum* (30) Fries gave various synonyms and author citations, including many of the authors previously mentioned in this summary. He did not pay much attention to spore characters in his descriptions of these fungi. Fries' later ideas are given below.

Brongniart (31, p. 93) included the "Tribes" Sphaeriacées (including Lophium) and Phacidiacées in the Hypoxylées.

Greville (32, Vol. II, pl. 167) in 1825 gave excellent plates of several Hysteria. He figured, for the first time, the asci and spores of certain forms. Greville placed Lophium with Hysterium in the Tribe Phacidiei of the order Phacidiacei. Acrospermum was included (Vol. VI) in the Gasteromycetes.

Chevallier (33) revived De Candolle's name Hypoderma, and transferred three species from that genus to his new genus Lophoderma. In 1826 (34, p. 432) Chevallier proposed the order Hysterineae to include Lophium, Hysterium, Lophodermium (as he spelled it here) and his new genus Schizoderma (? = Dichaena Fr.). This order he placed in his class Cheilomycei. Acrospermum he included in the Sclerotiaceae. Chevallier placed Leptostroma, Actinothyrium, Phacidium, Eustegia, Rhytisma, and Phoma in his order Rhytismaceae.

Schweinitz (35, 36) described for North America forty-three new species in Hysterium, Dichaena, Ostropa, Lophium, Glonium and Actidium, out of a total of sixty-seven species in these genera, which he considered to occur in his collections. Since he did not describe the asci and spores of these forms, much uncertainty still exists as to the nature of his species. Acrospermum was placed in his earlier work in Tremella. Schweinitz followed Fries' classification in his later work.

Duby (37, Vol. I, p. 718) followed Brongniart's classification. His later work is noted below.

Wallroth (38, p. xxix) included Xyloma, Phacidium, Tryblidium, Colpoma (a genus he described to include *Hysterium quercinum* Pers.), Lophium, Hysterium, Stictis, and Peziza in the Hymenomycetes of Fries.

Fries, in his *Epicrisis* (39, p. 1) proposed the name Discomycetes,

and in 1835 (40, p. 344) placed the Phacidiacei (including Glonium, Actidium, Hysterium, Phacidium, Rhytisma, and Leptostroma) in this class. The Dichaenei (including Actidium, Lophium, Ostropa and other genera) he left in the Pyrenomycetes, but noted "Transitum sistunt ad Discomycetes, sed discum verum non monstrant." (Actidium he included in both groups.) In 1849 (41, pp. 367, 400) he maintained the same general arrangement.

Mlle Libert (42) described Aylographum, and included it with Hysterium, Glonium, Actidium, etc., in the section Phacidiacei of the Xylomyci. Nees and Henry (43) and Rabenhorst (44) followed Fries.

Corda (45, 46) in 1842 gave the name Hysteriaceae to a family of his order Myelomycetes. The Hysteriaceae he subdivided into Stegiaceae (with Stegia), Hysteriaceae (including Ailographum, Hysterium, Sporomega Corda, and Lophium), Gloniaceae (with Hysterographium Corda and Glonium), Cliostomei (with Cliostomum and Actidium), and Phacidiei (including Phacidium, Heterosphaeria, and Rhytisma). Ostropa and Dichaena were placed in the Sphaeriacei. Spore characters were taken into consideration and several species were illustrated.

De Notaris (47) in 1844 considered spore characters further and made these the basis of classification of the Hysteriaceae. He divided the family into two sections: Phaeosporii, with Triblidium, Hysterium and Hysterographium, and Hyalosporii, with his Gloniopsis, and ten other genera. As the title indicates, these fungi were considered to be Pyrenomycetes.

Léveillé (48, p. 115) proposed Thécasporés as a division of Fungi, with subdivisions Ectothèques and Endothèques. The latter was divided into Tribes Rhegmostomés (including sections Hystériés and Cliostomés), Stégillés and Sphaeriacés.

Bonorden (49) followed Corda's classification.

Berkeley (50) proposed the name Ascomycetes for Léveillé's Endothèques. He (51, p. 379), however, followed Fries and other earlier workers in including Hysterium and other genera in the Phacidiacei.

Duby (52) in 1862 published his memoir on the Hysteriales. He considered that these fungi should be placed with the Pyrenomycetes for the following reasons: (1) the perithecia are horny or cartilaginous, (2) the perithecia are elliptical or linear, (3) they show a special method of dehiscence. He considered that the Hysteriales showed affinities with the Discomycetes (Pézizées) through Triblidium. Duby reviewed his previously stated ideas of their relations to, and differences from, the Lichens. He points out that Coemans had noted that the hymenium of the Hysteriales gives no reaction with iodine.

In discussing the value of diagnostic characters in the Hysteriales, Duby considered that de Candolle's separation on the basis of superficial or imbedded, and de Notaris' use of the colour of spores, were not of great value. Duby divided the Hysterineae into two sections, Lophiées and Hystériées, and made further subdivisions on the basis of dehiscence of asci. Under Lophiées he included Lophium as having dehiscent asci, and his new genera Ostreichnion and Mytilinidion as having indehiscent asci. In the section Hystériées he included Triblidium, Hysterium, Glonium, Aylographum, Hypoderma, Angelina, and Actidium with indehiscent asci, and Lophodermium, Sporomega, Coccomyces, Colpoma and Ostropa under dehiscent asci. Duby made a new genus Aporia, which he considered "anomalum et ambiguum." He gave generic and specific descriptions of the various forms, and illustrated twenty-five species. His division of genera on the basis of dehiscence of asci has not been followed.

The Tulasne brothers (53, Vol. I, p. 224; II, p. 258; III, p. 111) gave notes on several forms and illustrated one. In their classification they included the genera under Phacidiei. L. R. Tulasne (54) also recorded observations on the "spermagonia" of certain Hysteria.

Since 1865 there have appeared many works containing classifications, or revision of classification, of Fungi. Chevallier (33) had long since suggested a grouping which separated the Hysteriales from the Phacidiales, but Fries and many others had included the Hysteriales as a subdivision of the Phacidiales. Corda's suggestion of reversing the terminology and including Phacidiei under Hysteriaceae had not been followed. De Notaris (47) and Duby (52) had considered the Hysteriales as a distinct group, as did de Bary (55, pp. 190 etc.). Fuckel (56, p. 248) and Cooke (57, p. 750) followed Fries and included the Hysteriales with the Phacidiales in the Discomycetes. In a later paper Cooke (58) followed Duby.

Saccardo at first (59) followed in general the classification of Fries for these fungi, but later (60) carried de Notaris' (47) basis of classification on spore characters further and divided the Hysteriales into the Sections Hyalosporae, Didymosporae, Phragmosporae, Scolecosporae and Dictyosporae, placing them in the Pyrenomycetes. Spegazzini (61) elaborated further this idea in his "nova systematis carpologici dispositio," and classified the Hysteriales into Aplosporae, Didymosporae, Phragmosporae and Dictyosporae, each group being divided again into Hyalosporae and Phaeosporae. Thus, for example, the Aplosporae-Hyalosporae included the genera Aporia and Hypoderma. The Hysteriales he placed in the Angiothalamae (Pyrenomycetes). In 1883 (62) Spegazzini proposed the Hemihysteriaceae

with Morenoëlla, Schneepia and Hysterostomella, and he has added also other genera and species to the Hysteriales from his South American collections. In the *Sylloge* (Vol. II, p. 721) Saccardo modified his and Spegazzini's classification, and made nine sections, using descriptive words with Hyalo- and Phaeo- as prefixes.

Gillet (63) included the Phacidiales but not the Hysteriales in his work on the Discomycetes. Ellis and Everhart (64) included the Hysteriales in their work on North American Pyrenomycetes, although they remark that they had not at first intended to do so.

In Engler and Prantl, *Pflanzenfamilien*, Schroeter (65, p. 2) included the Hysteriineae, but not the Phacidiineae, in the Pyrenoasceae, but further on (p. 142) he included both these orders in the Discomycetes. Lindau (pp. 265–278) described the group, dividing it into five families. He noted that "Die Hysteriineae bilden eine Mittelgruppe" connected with the Phacidiaceae on one side and Lophiostomataceae on the other, being also similar to the Graphideae.

Rehm (66) went over Duby's collection and revised various forms. In Rabenhorst's *Kryptogamen-Flora, Die Pilze*, Rehm (67) discussed relationships, placing the order Hysteriaceae as a separate group between the Discomycetes and Pyrenomycetes. He classified the genera into three families, Hysterineae, Hypodermieae, and Dichaenaceae, with an "Anhang," Pseudohysterineae Rehm, to include Acrospermum. Several genera previously included in the Hysteriales were removed to the Pezizales. Rehm at various times added to the knowledge of the group (68, 69).

Boudier (70) did not include the Hysteriales in his work on the Discomycetes but Schroeter (71) and Seaver (72) so classified them.

Clements (73) in 1909 used the term "hysterothecium" for the fruit body of the Hysteriales and Graphidaceae.

During the past few years fundamental changes in the arrangement of the Pyrenomycetes and Hysteriales have been proposed. Theissen and Sydow (74) included several genera, which had been included in Hemihysteriaceae or Hysteriaceae, in their work on the Dothideales, and von Hoehnel (75) in 1918 removed several genera to the Phacidiales. In various of his *Mycologische Fragmente* von Hoehnel has given critical notes on genera of the Hysteriales. These notes of his and the views of other workers were summarized, and the results of further study were given, in his discussion "Ueber die Hysteriaceae" (76). Von Hoehnel pointed out that the group had come to be very unnatural. He considered the Hysteriaceae to be

Lophiostomaceae with more or less drawn-out perithecia (hysterothecia) and classified them with the Pyrenomycetes. He believed that these two families form a natural group, for which he proposed the name Hysterostomaceae.

Of the fifty-seven or more genera which had fallen into the Hysteriales, von Hoehnel decided that only ten of them really belong there. He gave a key for these genera, and separated out a new genus (or at least sub-genus) Psiloglonium, for species of Glonium without subiculum.

The disposition von Hoehnel would make of the genera to be removed from the Hysteriales may be indicated as follows:

To the Phacidiales (see also (75)): Schizothyrium, Hypoderma, Hypodermella, Hypodermopsis, Lophodermellina, Nymanomyces, Lophodermium, Lophodermella, Aldona, Colpoma, Hysteriopsis, Sporomega and Synglonium.

To the Ostropaceae (in the Discomycetes); Ostropa and Robergea; he would include these genera in a group with Stictis, Vibrissea, Schizoxylon, etc.

To the Triblidiaceae (formerly placed in the Phacidiales); Pseudographis, Rhytidhysterium, Tryblidiella, Tryblidium, and Delphinella (= Pleodothis and Pleoglonis).

To the Dothideales (see also (74)): Cycloschizon, Dielsiella, Hysterostomella, Mendogia, Parmulariella, Parmularia.

To the Lembosiaceae: Actidium, Aylographum (= Lembosiopsis), Hadotia, Morenoëlla, Lembosia, Lembosiella.

To the Sphaeriaceae: Delpinoëlla, Erikssonia, Graphyllium (= Clathyospora), Merillopeltis, and Schizacrospermum.

To the Patellariaceae: Baggea and Hysteropatella.

To the Cenangiaceae: Angelina.

To the Sordariaceae: Acrospermum.

To the Lichens: Phragmographium.

To be discarded: Aporia.

Table I includes the genera left by von Hoehnel in the Hysteriales, and gives the geographical distribution of the species so far as listed in the various volumes of Saccardo's *Sylloge*. In this table "Asia" includes the adjacent islands. It may be seen that there are a total of one hundred and thirty-two species listed as occurring in Europe, and one hundred and eleven in North America. Saccardo had not, of course, included data from various lists of fungi for different regions; there are for example many more than twelve species common to both Europe and North America. It must also be remembered that several species included by Saccardo have been or will be shown to be only synonyms.

The recent tendency to remove a number of genera from the Hysteriales merely indicates that the order had long remained

without comprehensive and comparative study. It still remains to be seen whether such radical re-adjustment as von Hoehnel proposed best fits a logical arrangement of the Ascomycetes; the intermediate position of the Hysteriales makes their study of high importance.

The scattered references to the parasitism of members of the Hysteriales have not been included in this paper, but it should

Table I. *Distribution of species according to Saccardo.*

	N.A. only	Eur. only	N.A. & Eur.	S.A. only	Asia only	Austral. & N.Z.	Africa only	Miscellaneous	Total
Dichaena	4	1	2	—	—	—	—	—	7
Farlowiella	—	1	1	—	—	—	—	—	2
Gloniella	5	21	—	12	6	2	—	1 doubtful	47
Hysterium	37	24	2	11	8	—	1	1 Eur., Asia and N.A. 1 Eur., S.A. and N.A.	85
Gloniopsis	7	19	1	3	1	2	—	2 doubtful	35
Hysterographium	18	11	5	10	3	—	4	1 N.A., Eur. and Austral. 1 N.A., Eur., Afr. 1 N.A. and Asia	54
Glonium	14	18	—	10	—	2	—	1 N.A. and S.A. 3 Eur. and S.A.	48
Bullardiella	—	1	—	—	—	—	—	—	1
Mytilidion	3	12	—	1	—	—	—	—	16
Ostreion	1	—	—	—	—	—	—	—	1
Lophium	4	5	1	1	—	—	—	—	11
Total	93	113	12	48	18	6	5	12	307

be noted that Tubeuf (77) succeeded in growing *Lophodermium pinastri* in pure culture. Brefeld had previously failed to culture a species of Lophodermium. Tubeuf did not succeed in producing infection on pines by artificial inoculations from his culture.

Summary.

The members of the Hysteriales were at first confused with lichens, certain of which they resemble. Early workers inevitably drew up vague descriptions of these fungi. The name of the group is based on the genus Hysterium, which was first used by Tode. The type species is *H. pulicare*, the name of which can be traced back to Lightfoot. Persoon succeeded in bringing a number of similar forms together, largely by an inclusive interpretation of the genus Hysterium. Chevallier first used the name "Hysterineae" for an order, and he also separated into another order certain genera now included in the Phacidiales. This work has been generally overlooked in considering the beginning of a separate grouping of these fungi. Corda used the family name Hysteriaceae to include also the Phacidiales; most

other workers, down to the middle of the 19th century, including Fries, placed the genera now classified in the Hysteriales with the Phacidiales. The genera came finally to be distinguished principally on the basis of the colour, shape, and septation of spores.

There has always been confusion in the use of names to distinguish orders, sub-orders, families, etc.; indeed, the same name is sometimes used by a writer in two senses: see Corda's arrangement. Several mycologists (Saccardo, Spegazzini, von Hoehnel (76), and others) include all the genera in one family; if von Hoehnel's contentions that Dichaena should be placed in a separate family, and that the Hypodermataceae, Ostropaceae, and Acrospermaceae all belong elsewhere, should be followed, only the family Hysteriaceae is left of those now usually (64, 67, 71) included in the order.

The Hysteriales have been placed in the Pyrenomycetes and Discomycetes about equally commonly, which indicates clearly that they form a transitional group; Rehm (67) compromised by placing them intermediately. Certain genera have likewise undergone a great amount of shuffling; the most conspicuous example being Acrospermum (which Tode (13, p. 8) described as "Fungus simplicissimus"!), which has been placed, as noted herein, in Clavaria, Sclerotiaceae, Tremella, Phacidiales, Hysteriales, Pseudohysterineae, Sordariaceae and recently by Riddle ((78) agreeing with Ellis and Everhart) in the Hypocreales. The most recent tendency is to reduce considerably the number of genera included in the Hysteriales.

Pycnidial stages, attributed to the Hysteriales, have been noted by certain workers (54, 04); these are usually hysterioid in nature, and include Leptostroma and Psilospora.

Many workers not mentioned herein have described species, extended the knowledge of the distribution, or added other data regarding the Hysteriales. It is obvious, however, that the group is still in need of comparative and synthetic study.

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NOTE.

A DIE-BACK IN SUSSEX.

Boughs of apple, Winter Pearmain and Lane's Prince Albert, affected with a bark disease have been forwarded to the Ministry by Mr John Goaman, one of the Ministry of Agriculture's Inspectors from the south-western, or Chichester, district of Sussex. This disease was considered by the growers to be causing a die-back and the trees have been cut back and the affected boughs burnt. The fungus present was in a pycnidial stage, the pycnidia globose, depressed, with a thick wall and set in a purplish mycelium, and with large hyaline spores of a Macrophoma type.

The fungus corresponds with New Zealand specimens of *Diplodia Griffoni* Sacc. & Trav.

Diplodia Griffoni Sacc. & Trav. Sacc. Syll. XX, p. 1228 (1911) and XXII, p. 994 (1913).

Pycnidia parasitic, somewhat large, scattered or grouped, simple, or divided into loculi; spores for a long time hyaline, thick walled, elliptic oblong, somewhat irregular, with granular contents 20–30 μ × 10–13 μ becoming ovoid and ellipsoid, uniseptate, 22–25 μ × 10–14 μ, fuliginous and quite smooth.

This fungus disease was first described from France (Diplodia sp. Griffon et Maublanc, Bull. Soc. Myc. France, 1910, p. 314, T. xiii et T. xiv) and occurs also in New Zealand as a bark parasite on apples. I am indebted to Mr Grove and Miss Wakefield for their assistance in identification.

M. L. ALCOCK,
Ministry of Agriculture,
Pathological Laboratory.

WILLIAM BERIAH ALLEN.

(1875—1922.)

By Carleton Rea.

William Beriah Allen was born at Benthall, Shropshire, in 1875. He was the only son of William Allen and Julia Caroline (née Lopez) and was educated at Ellesmere College, subsequently proceeding to Hertford College, Oxford. Unfortunately his father required his assistance in business and his career at the University was determined before he had completed the full number of terms, but doubtless if he had he would have graduated with high honours. His father was a potter by profession and after his death the son carried on the Benthall Pottery works and also developed a very prosperous foreign china and glass trade. Brought up in the beautiful country surrounding his home Allen in early life took a great interest in natural history and soon acquired a fair knowledge of the plants and birds of the district. He was afterwards attracted by the fungi, and it was as a student of these that I first made his acquaintance at the Hereford foray of the British Mycological Society in 1902. He had been advised by the well-known Shropshire mycologist, William Phillips, to attend this meeting but it is a somewhat curious fact that Phillips really tried to dissuade him from mycology. From this time onwards it was my privilege to rank him as one of my best and dearest of friends, and together we enjoyed many forays in search of our beloved fungi. Allen was more especially interested in the Basidiomycetae, Discomycetae and Mycetozoa and in all of these groups he made some very interesting discoveries and additions to the British Flora. He was a keen collector, quick to detect minute differences and well acquainted with the works of Fries, Quélet and Lister. Monsieur René Maire named *Omphalia Allenii* in his honour at the Baslow foray in 1909, and Miss A. Lorrain Smith dedicated *Cudoniella Allenii* to him from specimens gathered in Shropshire in the spring of 1907. In 1909 he described in our *Transactions*, vol. III, p. 92, the beautiful little violet Clavaria aptly named by him *Clavaria conchyliata* but which had been previously recorded by Saccardo under the uncouth name of *Clavaria Bizzozeriana*. He also found in my company the first British specimens of *Glischroderma cinctum* (Fuck.) Rea in Wyre Forest in 1909, of *Inocybe squarrosa* Rea near Worcester in 1915, of *Lycoperdon velatum* Vitt. at Aldenham, Shropshire in 1902, and of many others too

numerous to set out in a brief obituary notice. Allen was a very busy man and his engagements prevented him from attending many of the annual forays of our Society, and although on several occasions he was approached with a view to allowing his name to be proposed for the position of President of this Society we shall now deplore the fact that such an eminent mycologist was never elected to that position. He passed quietly away on the 20th of November, 1922, at the early age of forty-seven and was laid to rest in the pretty churchyard at Benthall three days afterwards, when it was my privilege to attend his funeral and pay my last tribute of respect and affection on behalf of the British Mycological Society and myself. All our members will deplore the loss to science and our society in particular by the death of such an able and distinguished mycologist and tender their heartfelt sympathy to his sorrowing widow.